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ADRP
AITRL
Akt1
Alpha-Feto Protein (AFP)
Alpha-Galactosidase A
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Angiostatin K1-3
Annexin-V
apo-SAA
Apolipoprotein A-1
Apolipoprotein E2
Apolipoprotein E3
Apolipoprotein E4
APRIL
Artemin
ATF2
Aurora A
Aurora B
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BAFF Receptor
BCA-1 / BLC / CXCL13
BCMA
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BD-2
BD-3
BDNF
Betacellulin
Bivalirudin
BMP-2
BMP-4
BMP-6
BMP-7
BMP-13
sBMPR-1A
Brain Natriuretic Protein
BRAK
Breast Tumor Antigen
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CSL2 Peptide
C-10
C-Reactive Protein
C-Src
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Calbindin D-28K
Calbindin D-29K
Calmodulin
Calcitonin Acetate
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CD95 / sFas Ligand
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CREB
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CTGFL / WISP-2
CTLA-4 / Fc
CXCL16
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COVER

Viewed from the inside of a fractured rice leaf, cells of the rice pathogenic bacterium *Xanthomonas oryzae* pv. *oryzicola* invade through a stoma. *Xanthomonas* species inject host cells with unusual DNA binding proteins called transcription activator–like (TAL) effectors to up-regulate genes important for infection. Two studies in this issue (pages 1501 and 1509; related Perspective, page 1491) decipher TAL effector target specificity and show that new specificities can be engineered.

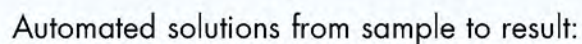
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- 1501 **A Simple Cipher Governs DNA Recognition by TAL Effectors**
M. J. Moscou and A. J. Bogdanove
Xanthomonas bacteria use an amino acid-based code to target effector molecules to specific DNA sequences.
 >> *Perspective p. 1491; Research Article p. 1509*

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C. Jørgensen et al.
 A proteomic strategy elucidates signaling networks between cells communicating through ephrin proteins and their receptors.
- 1509 **Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors**
J. Boch et al.
 Artificial effectors with new specificities have been constructed that mimic proteins injected into plant cells by pathogens.
 >> *Perspective p. 1491; Brevia p. 1501*

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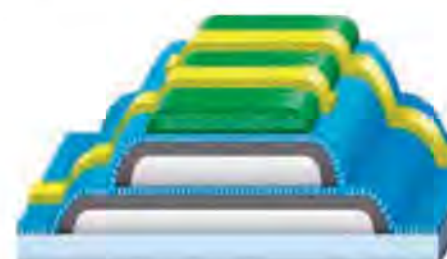
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 >> *Perspective p. 1490*
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- 1525 **Evolution of Organic Aerosols in the Atmosphere**
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 >> *Science Podcast*
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 >> *News story p. 1470*
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C. Louichaoen et al.
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A. H. Williams et al.
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 >> *Perspective p. 1494*
- 1554 **Norbin Is an Endogenous Regulator of Metabotropic Glutamate Receptor 5 Signaling**
H. Wang et al.
 The protein Norbin regulates accumulation of a neurotransmitter receptor in mouse brain cell membranes.

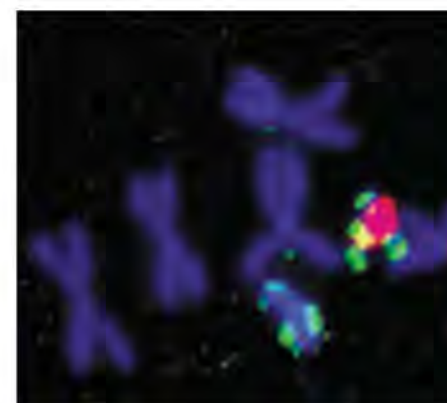
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Adaptive Evolution of Pelvic Reduction in Sticklebacks by Recurrent Deletion of a *Pitx1* Enhancer

Y. F. Chan et al.

Loss of a tissue-specific enhancer explains multiple parallel losses of the pelvic girdle in stickleback populations.
10.1126/science.1182213

Tuberculous Granuloma Induction via Interaction of a Bacterial Secreted Protein with Host Epithelium

H. E. Volkman et al.

Epithelial cells play a role in tubercular granuloma formation and mycobacterial virulence.
10.1126/science.1179663

Global Analysis of Short RNAs Reveals Widespread Promoter-Proximal Stalling and Arrest of Pol II in *Drosophila*

S. Nechaev et al.

The initially transcribed sequence plays a key role in inducing polymerase stalling.
10.1126/science.1181421

Iapetus: Unique Surface Properties and a Global Color Dichotomy from Cassini Imaging

T. Denk et al.

10.1126/science.1177088

Formation of Iapetus' Extreme Albedo Dichotomy by Exogenically Triggered Thermal Ice Migration

J. R. Spencer and T. Denk

Thermal migration of water ice explains the observed color asymmetry of Saturn's unusual moon, Iapetus.
10.1126/science.1177132

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RESEARCH ARTICLE: Only a Subset of Met-Activated Pathways Are Required to Sustain Oncogene Addiction

A. Bertotti et al.

Cells addicted to different oncogenic receptor tyrosine kinases develop common downstream mechanisms to sustain malignancy.

RESEARCH ARTICLE: Delivery of microRNA-126 by Apoptotic Bodies Induces CXCL12-Dependent Vascular Protection

A. Zernecke et al.

Apoptotic endothelial cells release microRNA-containing microvesicles to modulate the responses of neighboring cells and reduce atherosclerosis in mice.

REVIEW: Cracking the Phosphatase Code: Docking Interactions Determine Substrate Specificity

J. Roy and M. S. Cyert

Studies of the motifs that guide interactions between phosphatases and cellular proteins provide insight into phosphatase selectivity.

JOURNAL CLUB: To Be $\gamma\delta$ or Not to Be $\gamma\delta$? Signaling Pathways in $\alpha\beta$ Versus $\gamma\delta$ T Cell Maturation

J. K. Archbold

The role of Notch signaling in human T cell maturation is substantially different from that in the mouse.

NETWATCH: Domain Club Browser

Proteomic analysis organizes proteins into domain clubs that share similar domain composition; in Protein Databases.

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T. Luchian

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R. Sikorski and B. Yao

Predictive biomarkers can be integrated into phase III clinical trials by a variety of approaches.

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M. Jagodic et al.

Rat genetics implicates a new gene in the etiology of multiple sclerosis in European populations.

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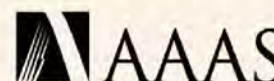
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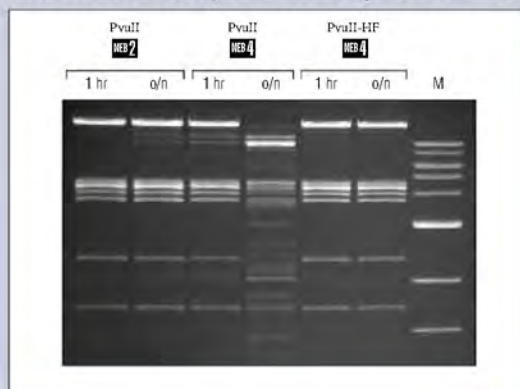
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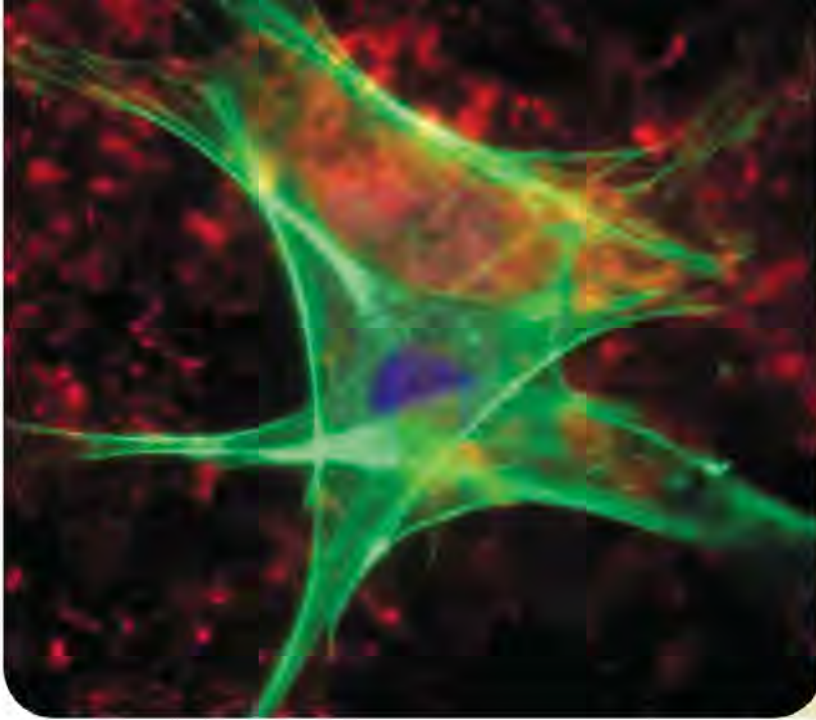
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<< Dissecting Ephrin-Receptor Interaction

Ephrins are transmembrane proteins that bind ephrin receptors on adjacent cells, leading to propagation of biochemical signals within both cells. *Jørgensen et al.* (p. 1502) devised a way to use differential isotopic labeling to distinguish cells engineered to express either the receptor or the ligand and to monitor bidirectional signaling events by mass spectrometry of the labeled peptides when the cells were mixed together. Signaling networks were constructed, and the information processing by the two interacting cell types was modeled. Changes in signaling within cells expressing just the ligand (ephrin) caused changes in the signal processing during the adjacent cell's response to binding of the ephrin receptor.

TAL Order

Xanthomonas bacteria attack their plant hosts by delivering their own transcription-activator-like (TAL) proteins into the plant cell nucleus and alter the plant's gene regulation (see the Perspective by *Voytas and Joung*). *Moscou and Bogdanove* (p. 1501, published online 29 October; see the cover) and *Boch et al.* (p. 1509, published online 29 October) have now discovered how the similar but not identical repeats in the TAL proteins encode the specificity needed for the proteins to find their targets. Each repeat is specific for one DNA base pair, a specificity encoded by hyper-variable amino acid positions. Combining several repeats with different amino acids in the hyper-variable positions allowed the production of new effectors that targeted new DNA sites.

Microquasar Spotted

Microquasars are binary star systems where a normal star sheds matter onto a neutron star or a black hole, generating x-ray radiation and jets of material moving at relativistic speeds. Microquasars have proved difficult to detect in high-energy gamma rays (> 100 megaelectron volts). Using the Fermi Large Area Telescope, *Abdo et al.* (p. 1512, published online 26 November; see the Perspective by *Bignami*) now report the detection of variable gamma-ray emission from the microquasar Cygnus X-3. The gamma-ray flux is modulated at the orbital period of Cygnus X-3, and its variation is correlated with the radio emission originating from the microquasar's relativistic jets.

An Odd Sort of Revolution

Joseph Hooker was the director of the Royal Botanic Gardens at Kew, London, when Charles Darwin and Alfred Wallace were presenting their

ideas about evolution by natural selection. Hooker was a good friend of Darwin's and an ardent ally of evolutionary thinking, who came to realize that natural selection would have little impact on the taxonomist's endeavor. *Endersby* (p. 1496) reviews taxonomic practice in the 19th century, arguing that the concept of evolution was almost a side-show in the energetic debate about whether to group varieties into single species or whether to divide species into endless varieties. On the one hand, Hooker was a "lumper," who found it hard to tolerate the thought of species constantly emerging, because it hindered his analysis of global patterns of species richness. On the other hand, Darwin's vision reconciled both modes of classification in revealing the genealogy of life on Earth.

Quick Spin Flips

Quantum computation holds the tantalizing promise of vastly improving the efficiency of traditional computers. Among the many solid-state candidates for storing and manipulating quantum information, nitrogen vacancy centers in diamond are especially attractive because they can be used at room temperature and stay operational for milliseconds at a time. To use this coherence time efficiently, it is important to achieve fast manipulation of the spins in the system. *Fuchs et al.* (p. 1520, published online 19 November; see the Perspective by *Gerardot and Öhberg*) used pulses of strong microwave magnetic field to probe the dynamics of single spins in a nitrogen vacancy center. In this "strong-driving" nonlinear regime, extremely quick spin flips of less than a nanosecond in duration were observed, offering the possibility that up to a million operations could be performed on a single spin during its coherence time.

Early Dinosaur Discovery

Our understanding of the evolution of early dinosaurs is hampered by limited material, especially compared to the many Jurassic and Cretaceous samples. *Nesbitt et al.* (p. 1530) provide a complete view of a Late Triassic theropod based on several nearly complete skeletons from New Mexico. The dinosaur elucidates the likely relationships between early theropods and shows that some prominent features were already derived by this time. Comparison among Triassic dinosaur fauna and



other early species suggests that Triassic North American fauna were diverse but not endemic, perhaps arising from earlier migrants from South America.

Gas Leak Inspection

The solid portion of Earth was formed from accretion of material and debris formed in the primitive Solar System. Earth's early evolution included the differentiation of its interior and the development of a primordial atmosphere. Heavy noble gases in the atmosphere could have been acquired during the initial accretion process or may have accumulated later through gravitational volatile capture. *Holland et al.* (p. 1522) show that Kr and Xe trapped in the upper mantle have isotopic signatures characteristic of early Solar System material similar to meteorites rather than those of the modern atmosphere and oceans. Thus, noble gases trapped within the young Earth did not contribute to Earth's later atmospheric composition.

Continued on page 1457

Congratulations to Elizabeth H. Blackburn **for her Nobel Prize in Physiology or Medicine 2009**

with Carol W. Greider and Jack W. Szostak.

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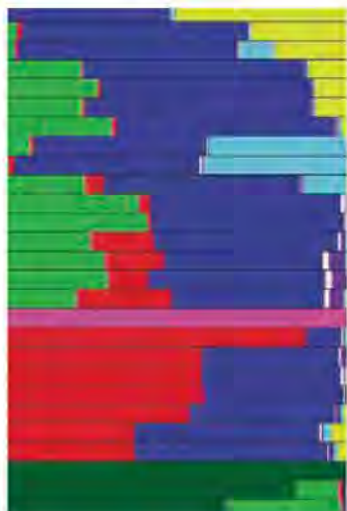
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Framework for Change

Organic aerosols make up 20 to 90% of the particulate mass of the troposphere and are important factors in both climate and human health. However, their sources and removal pathways are very uncertain, and their atmospheric evolution is poorly characterized. **Jimenez *et al.*** (p. 1525; see the Perspective by **Andreae**) present an integrated framework of organic aerosol compositional evolution in the atmosphere, based on model results and field and laboratory data that simulate the dynamic aging behavior of organic aerosols. Particles become more oxidized, more hygroscopic, and less volatile with age, as they become oxygenated organic aerosols. These results should lead to better predictions of climate and air quality.

Dissecting Amyloid Formation

Amyloid fibrils are associated with clinical disorders ranging from Alzheimer's disease to type II diabetes. Their self-assembly can be described by a master equation that takes into account nucleation-dependent polymerization and fragmentation. **Knowles *et al.*** (p. 1533) now present an analytical solution to the master equation, which shows that amyloid growth kinetics is often limited by the fragmentation rate rather than by the rate of primary nucleation. In addition, the results reveal relationships between system properties (scaling laws) that provide mechanistic insight not only into amyloid growth, but also into related self-assembly processes.



Patterns of Early Migration

In order to gain insight into various migrations that must have happened during movement of early humans into Asia and the subsequent populating of the largest continent on Earth, the **HUGO Pan-Asian SNP Consortium** (p. 1541) analyzed genetic variation in almost 2000 individuals representing 73 Asian and two non-Asian populations. The results suggest that there may have been a single major migration of people into Asia and a subsequent south-to-north migration across the continent. While most populations from the same linguistic group tend to cluster together in terms of relatedness, several do not, clustering instead with their geographic neighbors, suggesting either substantial recent mixing among the populations or language replacement. Furthermore, data from indigenous Taiwanese populations appear to be inconsistent with the idea of a Taiwan homeland for Austronesian populations.

An Innervative Small RNA

Amyotrophic lateral sclerosis (ALS) is a relentless disease characterized by progressive degeneration of motor neurons that control muscle movement, leading to muscle atrophy and paralysis. **Williams *et al.*** (p. 1549; see the Perspective by **Brown**) show that a small noncoding RNA that is selectively expressed in skeletal muscle, miR-206, senses motor neuron injury or loss and helps ameliorate resultant muscle damage by promoting regeneration of neuromuscular synapses. Expression of miR-206 was dramatically induced in a mouse model of ALS, and when this RNA was removed from mice by genetic manipulation, the disease progressed at a faster rate. The salutary effects of miR-206 appear to be mediated through a signaling pathway in muscle cells involving histone deacetylase 4 and a fibro-blast growth factor modulator, activation of which leads to release of factors that promote nerve-muscle interactions.

Norbin Knockout

Metabotropic glutamate receptors (mGluRs) are critical neurotransmitter sensors implicated in central neuronal functions like learning and memory and in diseases of the nervous system. **Wang *et al.*** (p. 1554) searched for proteins that interact with mGluR5a and identified a previously unrecognized component of the receptor signaling complex. The protein Norbin directly interacted with the receptor. Loss of Norbin in mice or cultured cells showed that it is necessary for the accumulation of mGluR5a in the cell membrane, for normal modulation of synaptic plasticity, and for some behavioral responses.

CREDIT: JIN ET AL.

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Harmonizing Global Science

EVERY MAJOR PROBLEM FACING MODERN SOCIETY NOW HAS A SCIENCE AND TECHNOLOGY COMPONENT—either as a cause or cure—whether it's energy and the environment, access to water and fertile land, the spread of infectious diseases, or sustaining a viable economy. Although every societal problem has unique regional characteristics that require attention, there are sufficient implications across regions for which only globally coordinated efforts will be successful. The recent assessments of the Intergovernmental Panel on Climate Change and their impacts on public and policy-maker perceptions provide one example of successful cooperation on a near-global scale. The betterment of humankind depends on a deliberate move from being an international community of scientists to being a truly global community.

As more countries have invested in science and technology to advance their societies, high-quality science is increasingly being carried out in every part of the world. The scientific enterprise has become highly collaborative both within and across countries. These trends present great opportunities and increasing obligations for the scientific community to contribute to solving society's major problems. But efforts will be successful only if the community can function in a much more globally integrated way.

Becoming global can only happen if the differences among national scientific communities are reduced. For example, there is substantial variation in the norms and standards that govern the work of scientists in different countries. Effective collaboration requires harmonizing these standards of conduct so that scientists can work together with full trust and confidence. Consider the work of the International Society for Stem Cell Research, which has been striving as a community to develop global guidelines for embryonic stem cell research so that biological materials developed in one nation can be shared with others. Similar concerns apply to other policies concerning the conduct of science, such as those regarding the use of human subjects, animal welfare, or work on genetically modified organisms. Harmonizing norms and standards may be the most pressing need for successful globalization. But disparate national intellectual property rules and regulations can also deter international cooperation, as can differing publication and information access policies.

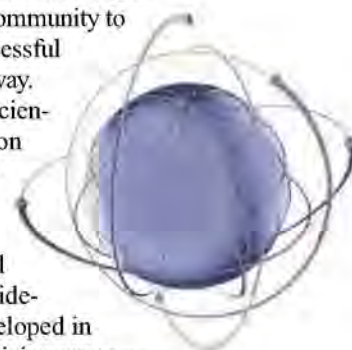
The heavily nationalistic funding policies of some wealthier countries and regions that make it difficult to support noncitizen students or to fund science conducted in other countries raise particularly difficult barriers to effective global collaboration. There is also the problem of daunting and widely varying administrative policies. A recent study by the U.S. Federal Demonstration Project showed that U.S. researchers spend 42% of the time allocated to research on administrative tasks. Add to this the need to meet the diverse bureaucratic requirements of different nations, and the burden on the global scientific community becomes truly excessive.

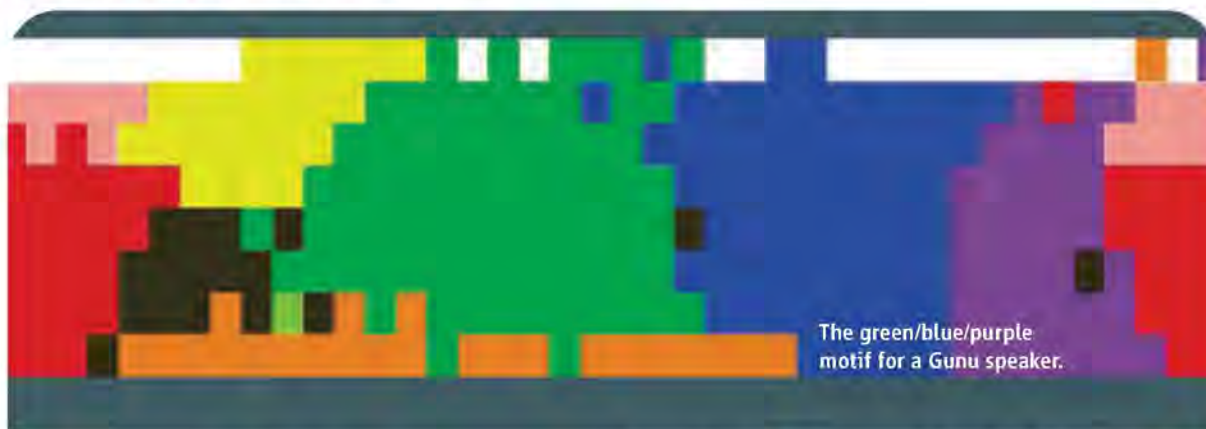
Creating greater uniformity across countries to reduce such deterrents will require both individual and institutional leadership. Perhaps regular international gatherings, such as the annual Science and Technology in Society Forum in Japan, the annual American Association for the Advancement of Science (AAAS) meeting, or the biannual World Science Forum in Hungary, could dedicate a major part of their time to working on these issues. However, globalizing science will require sustained efforts throughout the year, of a type not normally associated with these meetings. International scientific organizations could take the lead in efforts to harmonize overarching ethical norms and standards or intellectual property policies. Both public and private funders and policy-makers must be brought into the process early to make these endeavors successful.

The widespread increase in scientific activity throughout the world reflects great confidence in science's ability not only to reveal the nature of the natural world but also to contribute to the betterment of humankind. To exploit its full potential power, however, those involved in science and technology must become better able to function as a truly global community.

— Alan I. Leshner and Vaughan Turekian

10.1126/science.1184624





PSYCHOLOGY

That Which We Call a Rose

There is great diversity in how colors are represented among the languages of the world, but there are common categories into which colors tend to be grouped. Lindsey and Brown have explored how those categories are pieced together into naming systems and how those systems vary both across and within languages. They analyzed data from the World Color Survey; over 2000 individuals, representing over 100 mostly unwritten languages from nonindustrialized societies, provided names for the Munsell set of 330 color samples. Following on earlier work, they looked for categories into

which each individual grouped similar colors and identified 11 clusters into which color space can be divided, regardless of language. But in spite of this universal glossary of basic color terms, the ways in which these clusters mapped onto color space—their borders and distinguishing categories—was not uniform across individuals. There were three to six distinct naming systems, or motifs, that accounted for how individuals organized categories in color space. These motifs occur in languages spread across the globe, suggesting shared underlying mechanisms. Within many languages, however, multiple motifs were used, reflecting variations in how individuals modify their language. — BW

Proc. Natl. Acad. Sci. U.S.A. **106**, 19785 (2009).

ASTRONOMY

Capturing a Butterfly

Stars radiate because thermonuclear reactions in their cores transform hydrogen into helium. For most of their lives, radiation pressure balances gravity, preventing expansion or collapse. Once hydrogen starts to run out, however, the core contracts rapidly, leading to a temperature increase and an expansion of the star's outer layers: The star becomes a red giant. Helium fusion then starts, leading to instabilities that provoke ejection of most of the star's atmosphere. The ultraviolet radiation from the now exposed, hot surface of the star ionizes its surroundings and drives a fast outflow into the circumstellar material, heating it up and making it glow as a planetary nebula. Sometimes the central dying star may be hidden by the nebula itself and by a circumstellar disk of gas and dust. Such is the case with NGC6302,



also known as the Butterfly Nebula because of its bipolar morphology. Its long-elusive central star has now been detected by Szyszka *et al.*, who acquired optical images with the Wide Field Camera 3 recently installed on the Hubble Space Telescope. The star is situated in the center of the large-scale outflows, as expected, lying on the eastern edge of the thick dust lane. The data suggest that it has a high mass as compared to other planetary nebulae. — MJC

Astrophys. J. **707**, L32 (2009).

CELL BIOLOGY

A Sticky Business

Cohesin is a complex of proteins that binds to DNA. The name cohesin is derived from its function in maintaining the cohesion of sister chromatids after DNA replication; this ensures their faithful segregation into daughter cells during mitosis. The effects of mutations in cohesin proteins suggest that they may be involved in other nuclear events as well, such as gene expression.

Gard *et al.* have introduced mutations into yeast cohesin genes that correspond to those that are linked to the human developmental disorders Roberts syndrome and Cornelia de Lange syndrome. No effects on chromosome

cohesion were detected, but there were clear anomalies in nuclear organization: specifically, chromosome condensation, telomere arrangement, and nucleolus morphology. Furthermore, mutations in two of the cohesin proteins impaired the localization of a gene to the nuclear periphery that normally would occur upon transcriptional activation. Cohesin itself is highly conserved, and its role in nuclear processes may explain in part the abnormalities found in cohesin-linked human diseases. — HP

J. Cell Biol. **187**, 455 (2009).

CHEMISTRY

PC Squared

Both urea and thiourea groups, which comprise two nitrogen centers respectively flanking a C=O or C=S center, have recently proven useful in asymmetric catalysis of a wide range of organic reactions. Zhu *et al.* have modified this scaffold by replacing the central carbonyl with a longer, two-carbon bridge contained within a cyclobutene-dione moiety, and then applied the new framework to the asymmetric addition of phosphites to nitroalkenes. The strained central square geometry of this catalyst proved highly effective for promoting the P-C bond-forming reaction, affording high yields and enantiomeric excesses with both aryl- and alkyl-substituted

nitroalkenes. Reduction of the nitro group in these products would afford β -amino phosphonic acids, intriguing analogs of the more common amino (carboxylic) acids. — JSY

Angew. Chem. Int. Ed. 10.1002/anie.200904779 (2009).

CROP SCIENCE

Up and Down the Paddy

When staple crops such as rice are grown in soils naturally rich in toxic metals, containing the risk of sickness or even death from exposure becomes a top priority. In Asia, with large and often growing populations to feed, simply abandoning land with contaminated soils is not a viable option; water management strategies are instead employed to decrease the uptake of problematic elements while still promoting abundant rice yields. In rice uptake experiments using Japanese soils, Arao *et al.* found that two common water management strategies, flooding and aerobic treatment, have opposing effects on arsenic (As) and cadmium (Cd) levels in rice. In flooding conditions, significant amounts of As were incorporated into the rice grain and straw, whereas Cd primarily remained in the soil. Conversely, aerobic treatment increased Cd content in rice but caused very little As uptake. The variable effect is explained by changes in mineral solubility with changing soil redox potential: The oxidizing conditions of aerobic treatment convert insoluble Cd sulfide into the mobile sulfate, whereas reducing conditions from flooding increase arsenite mineral dissolution and hence As mobility. However, redox potential alone does not explain why the organic fraction of As in the soil is translocated more easily. Biological factors such as methyltransferase activity in rice may be responsible for that observation. — NW

Environ. Sci. Technol. 10.1021/es9022738 (2009).



MARINE BIOLOGY

Carbon Capture, No Storage

Sponges that inhabit coral reefs act as high-volume water filters, acquiring up to 90% of their daily carbon intake from dissolved organic matter, yet the biomass of these sponges remains constant. De Goeij *et al.* have resolved this discrepancy by analyzing cell turnover in the

sponge *Halisarca caerulea*, collected from coral reef cavities off the island of Curaçao. 5-bromo-2'-deoxyuridine (BrdU) is a thymidine analog whose incorporation into DNA is a measure of cell proliferation. Analyses of BrdU levels in sponge tissue showed that choanocytes—flagellated cells that line the internal passageways—were the sole type of proliferating cells, with an unusually short cell division cycle of 5 hours. The authors observed a massive amount of choanocyte shedding in the central canals, which may be analogous to the loss of epithelial cells in the gastrointestinal tract. The rapid turnover of choanocytes, each containing roughly 3 pg of carbon, helps to avoid clogging of the filter chambers and balances the sponge's carbon intake. — LC

J. Exp. Biol. 212, 3892 (2009).

CANCER

An Inflammatory Path to Cancer

Switching normal cells into a transformed phenotype that is characterized by uncontrolled growth is central to the development of cancer. Iliopoulos *et al.* describe an epigenetic switch:

a stable phenotypic change, retained through multiple generations in proliferating cells, which is not due to changes in DNA sequence. Immortalized cells from mammary epithelial tissue were transformed by overexpressing Src, a protein tyrosine kinase; activation of Src for just 5 min produced cells that adopted a transformed phenotype and maintained it for at least 12 generations. Activating Src led to an increase in activity of the transcription factor NF- κ B, a central mediator of inflammatory responses. A key target of NF- κ B

in this system was Lin28, an RNA-binding protein that inhibits the accumulation of the microRNA let-7. In turn, let-7 reduces levels of interleukin-6 (IL-6), an inflammatory cytokine thought to contribute to human cancers. IL-6 signals through its receptor to activate NF- κ B, thus creating a positive feedback loop. Disruption of any step in the loop resulted in loss of transformation. The authors note that the signaling events defined in this study are known to be associated with certain human cancers. Thus, the epigenetic switch they describe could allow a transient inflammation to produce a long-lasting cancerous effect analogous to mutation of a tumor suppressor gene or activation of an oncogene. — LBR

Cell 139, 693 (2009).

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Retro Science

In the early days of blood transfusion, a colleague of British scientist Robert Boyle transferred blood from a lamb to a man (left). Such practices were banned in the 1670s because of often-fatal immune reactions. This image is included in a new online exhibit of pathbreaking papers published by Britain's Royal Society, which turns 350 years old in 2010.

At the society's new Web site, trailblazing.royalsociety.org, history buffs can browse 60 papers along with brief introductions by experts. The oldest is a 1666 paper by Boyle suggesting experi-

ments transfusing blood—which was thought to have properties now ascribed to genes—among dogs. The most recent paper, from 2005, analyzes techniques for geo-engineering: altering Earth on a massive scale to curb climate change. Other works include Newton's revelation that white light is a blend of other colors, van Leeuwenhoek's discovery of bacteria in rainwater, a test of Einstein's theory of general relativity during the 1919 eclipse, and musical examinations of the 8-year-old Mozart.



Magic Mushroom

Yum yum. Looks like a marshmallow bar with pistachios and coconut. Actually, although not as tasty as it looks, it wouldn't hurt you to eat it. It's a new invention called EcoCradle: packing material based on the roots—mycelium—of mushrooms. The stuff sprouted from the brains of two Rensselaer Polytechnic Institute graduates, Eben Bayer and Gavin McIntyre. Noting how mushroom roots bind bits of forest detritus together, they have perfected a method of getting mycelium to grow around rice, cottonseed, hazelnut, and buckwheat hulls. The resulting material is popped into an oven to firm up. A cubic centimeter of the stuff contains

EcoCradle chunk.
A micrograph of mycelium (inset).



about 700 meters of mycelium, which gives it its strength. The inventors say it's totally biodegradable and no more costly than polystyrene, which now occupies about 25% of landfill space in the United States, according to the Environmental Protection Agency.

Nation of Flab

Obesity is edging ahead of smoking as a health hazard in the United States, say researchers at the National Bureau of Economic Research in Cambridge, Massachusetts.

A team led by Susan Stewart, a researcher who studies aging, has come up with some hard numbers on the skyrocketing problem: In the past 15 years, smoking has decreased by 20%, but the number of fat Americans has increased by 48%. By 2020, the team calculates that obesity will rob an 18-year-old of 0.7 years of life on average and 0.9 years of "quality of life."

The average gain for an individual from not smoking—0.3 years—is more than offset by the loss of more than a year from weight gain, the authors reported last week in *The New England Journal of Medicine*. Even if obesity increases level off to as little as 0.15% per year, they'll swamp overall gains from non-smoking by 2020.

THREE Q'S >>

Last week, Nobelist **Eric Kandel**, a neuroscientist at Columbia University, was interviewed on stage at Rockefeller University after a screening of a documentary on his life, *In Search of Memory*, as part of his 80th birthday celebration. Following is a condensed version of some of his remarks.



Q: You've said that the 20th century saw the merging of psychology and biology. What recent advances excite you?

Until quite recently, there has been very little hard data [on the effectiveness of psychotherapy]. In the last 20 years, people have done ... systematic studies that show that cognitive behavioral therapy is as good if not better than selective serotonin uptake inhibitors in moderate depression. Moreover, imagistic studies in obsessive-compulsive neurosis and depression have shown an abnormality in the brain. That's remarkable progress.

Q: Synaptic plasticity is a central concept in your work on memory. You've been working with *Aplysia* since 1962. What else do you think we can learn from these lowly snails?

With almost all kinds of synaptic changes, there is a parallel change in the excitability of nerve cells. For example, in *Aplysia*, a number of neurons fire spontaneously, in bursts. If you [stimulate] a bursting cell [synaptically], you can change its bursting activity for long periods of time [which implies plasticity not only in the synapse but the neuron itself]. This just blew me away. [But] I've never come back to it.

Q: What should we think about the rise of biotech and the mingling of the private and public sectors?

I was raised in an environment in which ... having any connection to the commercial realm ... was bad. And then, Genentech came along. My policy is that once a problem that I work on moves into a company I'm associated with, I don't work on it any more in the lab. It would be very awkward if the people in your lab felt that your approval of their research depended on the financial gain you would have from it.



ANIMAL RESEARCH

Rejection of Anthrax Study Kicks Up a Dust Storm in Oklahoma

A media maelstrom erupted last week over a primate research project that was rejected by administrators at Oklahoma State University (OSU) despite winning federal funding and approval from the university's animal care and use committee. The move triggered charges that the university had flouted the standard scientific and ethical review process to curry favor with a generous donor who is a vocal animal-rights activist. A university official strenuously denies that charge but says that fears of attacks from animal-rights extremists were a major factor in the decision.

The decision is unusual if not unprecedented, says Mark Lively, a biochemist at the Wake Forest University School of Medicine in Winston-Salem, North Carolina, and president of the Federation of American Societies for Experimental Biology. Lively says he knows of no other example of a university administration overruling its own animal care committee, let alone passing up an opportunity to receive federal research money.

The project aims to develop methods for studying anthrax infection and testing vaccines and treatments in baboons. It is one component of a larger, ongoing research effort led by Shinichiro Kurosawa, an immunologist at Boston University School of Medicine who worked previously at the Oklahoma Medical Research Foundation in Oklahoma City and has continued to collaborate with researchers there and at OSU. The National Institutes of Health agreed to fund the research, and after a lengthy review, OSU's Institutional Animal Care and Use Committee gave it a green light. But in October, Stephen McKeever, OSU's vice president for research, notified Kurosawa that university President Burns Hargis had decided that the project would not be allowed at OSU.

Kurosawa declined to comment, beyond writing in a brief e-mail message: "As guest scientists at OSU, we are obliged to follow their policies and it is unfortunate that we cannot fully complete our research there at this time." Colleagues say Kurosawa will now have to try to work out an agreement



Do not mix. Officials at OSU have decided to disallow a study of anthrax (*background*) in baboons, citing concerns about animal-rights extremists.

with one of the few labs in the country that have the capacity to handle both primates and infectious anthrax.

The issue gained attention last week when *The Oklahoman* newspaper picked up the story, and it quickly provoked a commotion in the blogosphere. In response, the university issued a statement saying that "The OSU administration determined that this research was not in the best interest of the university. The testing of lethal pathogens on primates would be a new area for OSU that is outside our current research programs."

Those sentiments seem at odds with the university's recent investment in a new biosafety level 3 laboratory on the Stillwater campus, where Kurosawa's research would have been conducted. When the lab opened in 2006, officials declared it would help position the university to bring in more federal money for research on biodefense and infectious disease. "OSU and the College of Veterinary Medicine have made a sustained investment in building the resources and infrastructure to do this kind of research, and now suddenly it's off the table," says Michael Davis, a veterinarian and physiologist at OSU and a member of the animal use committee that approved the anthrax project.

Researchers at OSU and elsewhere were

quick to speculate that Hargis's decision was influenced by the university's most prominent donors, billionaire T. Boone Pickens and his wife, Madeleine, an outspoken animal-rights activist. Pickens has donated \$458 million to the university in recent years, according to a September article in *The New York Times*. Madeleine Pickens caused a flap earlier this year when she stipulated that a \$5 million donation to OSU could not be used to benefit the veterinary school because of what she described as "barbaric" practices used in the surgical training of students. Faculty members protested that Pickens's allegations were based on faulty information, but the school ultimately changed its policy; it now bans euthanasia of animals used in teaching labs.

McKeever flatly denies that the Pickenses had a role in the decision to block the anthrax project. "We never had any discussions with them about this issue," McKeever says.

Hargis made the decision based on several factors, McKeever says: "The issue he was mostly concerned about was that he really did not want to attract controversy from the violent elements of various animal-rights groups. He did not want to put OSU in that spotlight and so unnecessarily distract from or interfere with current research."

The decision has caused anger, confusion, and concern among OSU researchers. "It certainly makes us look like a backwards, hill-billy state university," says Richard Eberle, a virologist at OSU who had planned to collaborate with Kurosawa. "I suspect OSU is going to be looked at a little more carefully as a potential research partner in the future." Eberle and others say they've received no clear indication from the administration about what kinds of research it now considers permissible. "The goalposts have definitely shifted," says Davis. "The problem is we don't even know where they've gone."

The statement issued by the university last week says: "the administration has simply decided that OSU will not have primates euthanized on its campus." Although that sounds like a categorical declaration of future policy, McKeever told *Science* that any future proposals involving primate euthanasia will be judged on a case-by-case basis: "We're in discussions with the faculty about establishing procedures by which we will review such proposals in the future."

—GREG MILLER

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Sunlight
to gasoline

1472



We are what our
ancestors ate

1478

CLIMATE CHANGE

Deforestation Moves to the Fore in Copenhagen

As the Copenhagen climate meeting unfolds this week, one key topic will be protecting the world's tropical rainforests. Deforestation is responsible for between 12% and 25% of the world's yearly greenhouse gas emissions. But the 1997 Kyoto agreement, which runs through 2012, didn't address the issue. Participating nations hope to correct that oversight in the next agreement. "It is likely that in Copenhagen the international community will commit to reducing the rate of deforestation by a specific amount, possibly 50% by 2020," predicts Frances Beinecke of the Natural Resources Defense Council in Washington, D.C.

Stopping deforestation is among the cheapest ways to reduce emissions, at an estimated cost of less than \$10 per ton of carbon dioxide. Last month, Brazil announced that it would voluntarily reduce its carbon emissions, most of which come from deforestation, by 36% to 39% by 2020 (*Science*, 27 November, p. 1175). But government officials are counting on an international system that compensates landowners, ranchers, and farmers for not cutting down trees.

A number of thorny issues must be resolved before that can happen. Last month in Barcelona, Spain, national representatives left dozens of key phrases in brackets in the draft text, indicating language still in dispute. "They didn't even decide whether they would aim for official treaty language or further official 'guidance' coming out of Copenhagen," says Kate Cecys of the Pew Center on Global Climate Change in Arlington, Virginia.

Negotiators call the issue Reducing Emissions from Deforestation and Forest Degradation (REDD). But just what shade of REDD will count is a matter of hot debate. Should developing countries get credit for reforestation, or is it enough to simply prevent the destruction of standing forests? Developed countries, which would presumably fund any such system, are leery of anteing up before they receive what they view as credible assurances from participating nations. Deciding what types of activities will be eligible for REDD funds "may be too much to sort out in the time we have," said Tracy Johns of the Woods Hole Research Center in Massachusetts.

Since 1997, scientists have improved their measurements of emissions from deforestation. That knowledge, along with a better understanding of what drives forest emissions and how to combine ground and satellite data, has helped to win over skeptical politicians. In 2003, the Intergovernmental Panel on Climate Change spelled out a step-by-step process to estimate land use, emissions, and uncertainties related to deforestation. The United States and other developed nations prefer those guidelines, but countries with large tropical forests haven't said whether they support such an approach to assess their carbon stocks. Another sticking point is whether countries will get credit for individual projects or instead be held accountable for comprehensive national commitments.

Compensating landowners or farmers for REDD could cost \$18 billion or more per year, and there is no consensus on how to raise the money. Brazil's federal government, for example, has generally preferred direct payments from an international fund. (Norway has pledged \$1 billion toward efforts in the Amazon, contingent on Brazil's actions on REDD.) But Brazil recently warmed to the idea of developed countries using carbon-credit offsets under an international system to pay for REDD projects. (The cap-and-trade bill passed last summer by the U.S. House of Representatives allocates 5% of emissions certificates for such credits.)

Other players are thinking locally. The state of Mato Grosso, Brazil, this week announced an arrangement in which U.S. companies operating under future emissions caps could purchase early carbon credits that would fund forest protection in the Amazonian state. "We want to take advantage of the momentum on REDD to get some programs in place," says forest scientist Daniel Nepstad of Woods Hole, who helped arrange the program.

—ELI KINTISCH



CREDIT: ANTONIO SCORZA/AFP PHOTO/NEWS.COM

Herd the call. Delegates in Copenhagen are expected to act on deforestation, often a side effect of ranching.

ANTHROPOLOGY

Chagnon Critics Overstepped Bounds, Historian Says

PHILADELPHIA, PENNSYLVANIA—The scene was familiar. Almost exactly 9 years ago this week, a packed session at the American Anthropological Association's (AAA's) annual meeting rancorously debated the inflammatory misconduct charges in journalist Patrick Tierney's book, *Darkness in El Dorado: How Scientists and Journalists Devastated the Amazon*. That 2000 session went

problematic were AAA's actions, she charged, "I can't imagine how any scholar feels safe" as a member.

The Yanomamö have become something akin to anthropological celebrities: A relatively large and non-Westernized indigenous group that still largely makes a living by hunting, foraging, and slash-and-burn agriculture, they became well-known through Chagnon's work. His book, *Yanomamö: The Fierce People* (1968), and documentaries launched a cavalcade of research on the Yanomamö, who have since been studied by as many as 50 anthropologists. From the beginning Chagnon's work attracted criticism, especially his view that warfare is a key building block of Yanomamö society. This attracted the ire of both opponents of evolutionary psychology and indigenous-rights activists, who charged that Chagnon's ideas were being used to justify taking Yanomamö land. Eventually, these criticisms helped to get Chagnon banned from research in both Venezuela and Brazil.

But Tierney's charges went well beyond the scientific. Although *Darkness* excoriated many anthropologists, the book focused on Chagnon, and especially a 1968 incident in which Chagnon and the celebrated late geneticist James R. Neel vaccinated Yanomamö and observed their immune responses. Tierney argued that the pair had never obtained informed consent and exacerbated or even caused a fatal measles epidemic.

AAA, apprised of the book's charges in September 2000, asked a commission led by former AAA President James Peacock for a confidential report—"inevitably meaning that Chagnon couldn't confront his accusers," Dreger said in an interview, and heading "down the path of violation of due process." Peacock, as he said at the AAA meeting last week, decided the charges had enough evidence to merit "an investigation." However, the AAA ethics code, adopted in 1998, forbids "adjudicat[ing] claims for unethical behavior," so AAA assembled a

task force to conduct an "inquiry."

The task force exonerated Chagnon and Neel of the epidemics charges (*Science*, 19 January 2001, p. 416) yet concluded that Tierney's allegations "must be taken seriously" and said Chagnon's work "had been damaging to the Yanomamö." In 2005, the AAA membership voted by a large majority to rescind the task force report, but it remained on the AAA Web site until September.

Despite the task force's conclusion, Dreger obtained an e-mail from the task force chair, former AAA President Jane Hill of the University of Arizona in Tucson, describing the book as "just a piece of sleaze." And task force member Janet Chernela of the University of Maryland, College Park, Dreger said, told her that "nobody took Tierney's book's claims seriously." The inquiry was conducted, Dreger charged, largely because AAA wanted to safeguard U.S. researchers' future access to the indigenous peoples in Latin America; they didn't want other anthropologists to become tarred with the same brush.

In an e-mail to *Science*, Chagnon said he had been "dumbfounded" to learn from Dreger that task force members had thought little of Tierney's work but "went ahead with their shameful witch hunt of Neel and me."

Invited to respond, Terence Turner of Cornell University, a longtime Chagnon critic, argued that last week's meeting was unfairly set up—he had 15 minutes to respond to what amounted to an hour of critique. Moreover, he

observed, Tierney's book did much more than attack Chagnon, and neither the AAA task force nor Dreger had addressed its discussion of other, putatively unethical work. Tierney did not respond to Dreger's inquiries and was not at the meeting. Reached by *Science*, he echoed Turner's point and defended his work against specific allegations.

As Dreger observed, many of the most bitter feuds in social science erupt over questions of "human identity." Because that question is central to anthropology—and because indigenous peoples are often involved in political struggle—battles are common. Yet AAA has made no institutional changes to better handle the next eruption, says Dreger, such as altering its code of ethics. "They've learned nothing," she said.

—CHARLES C. MANN



When worlds collide. Napoleon Chagnon (left) was attacked for his dealings with the Yanomamö people.

over its allotted time and dissolved into acrimony. On 2 December, the AAA annual meeting held another panel discussion on *Darkness*. It, too, was packed (though the room was smaller), filled with ethics charges and bitter, sometimes personal debate, and unable to finish on time, as arguments spilled into the hallways. Leaving the room, Robert Carneiro of the American Museum of Natural History in New York City told *Science*, "I don't know if this is ever going to end."

This year's meeting had a different slant on the ongoing fight over Tierney's book, which focused on anthropologists' treatment of the Yanomamö Indians of southern Venezuela and northern Brazil. Nine years ago, much of the meeting echoed Tierney's book in attacking Yanomamö researcher Napoleon A. Chagnon, now a professor emeritus at the University of California, Santa Barbara. This time around, most of the criticism was leveled at Chagnon's accusers and the AAA itself. In the 2 December session, historian Alice Domurat Dreger of Northwestern University's Feinberg School of Medicine in Chicago, Illinois, reported on her research into AAA's role in the affair, as part of a book on scientific controversies. So



Turning the tables. Alice Dreger criticized Chagnon's critics.



Supply and demand. Demand for the newly approved cell lines is expected to skyrocket.

STEM CELLS

NIH Approves First New Lines; Many More on the Way

At long last, federally funded stem cell researchers will soon have the access to human embryonic stem (ES) cell lines that they have been hankering for over the past 8 years.

Last week, the National Institutes of Health (NIH) named the first 13 lines approved for federal funding under President Barack Obama's revised policy—11 derived at Harvard University and two at Rockefeller University. NIH Director Francis Collins was expected shortly to approve 27 more; a further 68 are awaiting review. At a 2 December press conference, Collins said NIH expects submissions soon for at least 100 more. Thirty-one research grants, totaling \$21 million, have been on hold pending the official availability of the cell lines.

Last March, Obama announced he was deep-sixing the Bush Administration's stem cell policy, which limited federally funded researchers to 21 cell lines. Scientists had worried that, under exacting new requirements for informed consent by embryo donors, most of those 21 lines wouldn't make the grade. But NIH has fashioned a two-tiered vetting process that allows some flexibility in considering lines generated before 7 July 2009—the date the NIH guidelines were finalized—as well as lines from other countries where the informed-consent procedures may vary slightly.

But that doesn't mean NIH isn't being extremely fastidious about the process. At a 4 December meeting of NIH's Advisory Committee to the Director, its working group for human ES cell eligibility recommended that Collins approve 27 lines derived at Harvard. Harvard had submitted 28 lines for approval, but one line came from an embryo whose donors signed a consent form during a 7-month period when approval for the form by Harvard's Institutional Review Board had lapsed. Harvard researchers decided it was kosher given that the same consent form was

used before, during, and after the lapse. But the working group decided to reject that line. The approval process must be "beyond reproach," said working group chair Jeffrey Botkin, a pediatrician at the University of Utah School of Medicine in Salt Lake City.

Another issue is whether NIH should, in addition to vetting cells for funding eligibility, define restrictions on their use. Botkin pointed out that the "anonymization process" for donors means that there's no way to check whether they approve all the uses to which scientists put their genetic material. Participants decided that all cell populations from the 27 lines should be used only for purposes spelled out in the consent form: "to study the embryonic development of endoderm with a focus on pancreatic formation." Collins said NIH would establish a policy for how to handle such issues in the future.

Researchers holding sought-after lines must now figure out how to deal with the time and expense involved in distributing them. Ali Brivanlou of Rockefeller University in New York City said last week that he had already received about 50 requests for his lines, which he uses to study brain development. "We generated hundreds of independent tubes for each line," so they'll have plenty to give away, he says. George Daley of Harvard Medical School in Boston, who so far has received requests from more than a dozen investigators, says it's not going to be easy to satisfy the demand: "Our plan is to get the cells into the hands of a bank that will distribute over the long haul."

At the meeting, Collins said that "if it turns out this is a real barrier," NIH is prepared to consider setting up such a repository. "I would certainly welcome that," says Brivanlou. "It would take the burden of distribution and quality control to a central location, and there's no better place than NIH for that."

—CONSTANCE HOLDEN

ScienceNOW.org

From Science's Online Daily News Site

Lose Genes, Gain Weight

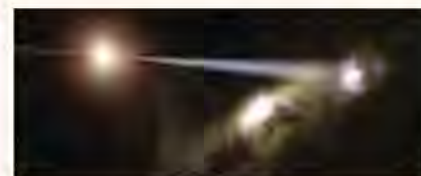
Obesity is a disease of excess, but a new study suggests that a few obese patients are actually lacking something—a piece of one of their chromosomes. The loss might remove a gene that helps the body manage blood sugar and appetite. <http://bit.ly/cmvappetite>

Why Your Older Brother Didn't Share

If you watch enough television, you'll witness what psychologists describe as birth order stereotypes. Take Alex P. Keaton of the 1980s U.S. sitcom *Family Ties*. Firstborn Alex was far more brash and competitive than his younger sisters, reading *The Wall Street Journal* while in high school, for example. Now scientists report that the stereotype is valid: Eldest children are less cooperative, trusting, and reciprocating than their siblings. <http://bit.ly/olderbrother>

An Introduction to Monkey Grammar?

It's not quite Shakespearean wordplay, but a species of African monkey can modify individual warning calls to produce novel meanings, according to new research. And because the wild monkeys tack the same sound onto the end of their calls, the authors speculate that they could resemble suffixes. But it's debatable whether the sounds serve a grammatical purpose like that in human language. <http://bit.ly/monkeygrammar>



The Quasar That Built a Galaxy

Which came first, the quasar or the galaxy? Astronomers have long believed that young galaxies feed the black holes at their centers until those black holes become quasars, which are incredibly massive and powerful sources of energy. But scientists have now found a quasar that's apparently churning out new stars in the absence of a host galaxy. The discovery suggests that quasars created at least some galaxies, not the other way around. <http://bit.ly/galaxybuilder>

Read the full postings, comments, and more on scienow.sciencemag.org.

BIOMEDICAL RESEARCH FACILITIES

Plans for London 'Cathedral Of Science' Unveiled

LONDON—Two years ago, as the world's economy was expanding, U.K. Prime Minister Gordon Brown announced plans for a mammoth new biomedical facility to house more than 1200 researchers in the heart of this booming city. Since then, the global financial crisis has derailed many ambitious projects, but the proposed £500 million-plus United Kingdom Centre for Medical Research and Innovation (UKCMRI) apparently remains on track. At a media briefing this week, backers affirmed their financial commitments to the project and unveiled architects' drawings and a "scientific vision" for the facility.

"There's been no dialing back" of ambitions, says Paul Nurse, the British Nobel laureate and head of Rockefeller University who was tasked with developing the facility's science plans. UKCMRI's "design has a simple aim: Keep Britain at the forefront of biomedical research in the world."

The proposed "cathedral of science," as UKCMRI's lead architect calls it, arose from a fight over the National Institute of Medical Research (NIMR) in Mill Hill. That aging but celebrated research center on the outskirts of

London has been slated for closure for years after its owner, the Medical Research Council (MRC), decided to move these labs downtown near hospitals and universities to promote translation of basic research into the clinic (*Science*, 4 February 2005, p. 652). Various relocation plans were fought over and abandoned until 2007, when MRC joined with several others—University College London (UCL) and the biomedical charities Wellcome Trust and Cancer Research UK (CRUK)—to propose UKCMRI (*Science*, 14 December 2007, p. 1704). "No one group can fund something this big," says developmental biologist Jim Smith, director of NIMR.

UKCMRI, which won't be ready until the end of 2014 at the earliest, will be built on land purchased for £85 million that sits behind the British Library and next to major train terminals, including one with high-speed rail access to the rest of Europe. Those transport links, as well as cutting-edge facilities, will enable UKCMRI to create an international hub of

Grand designs. More than 1200 scientists may work in this proposed building by 2015.



research, says Nurse. He envisions UKCMRI becoming a "feeder" for the rest of the United Kingdom by training early-career scientists but not keeping them. "This will be different. We will celebrate departures," says Nurse.

In addition to NIMR scientists, who tend to specialize in stem cells, infectious diseases, and immunity, UKCMRI will incorporate scientists from CRUK's London Research Institute (LRI), which has strengths in cellular and molecular biology and genetics. "It's a marriage made in heaven," says LRI Director Richard Treisman.

UCL, which plans to have about 150 of its own scientists at the new institute, will provide clinical access to its affiliated hospitals and to

GENE THERAPY

β -Thalassemia Treatment Succeeds, With a Caveat

Gene therapy researchers are approaching a key milestone: They appear to have controlled an inherited blood disorder called β -thalassemia that's more common than any disease treated so far with gene therapy. A young man who received new genes to repair blood cells no longer needs regular transfusions and, 2 years later, seems healthy. Describing this success last week to a U.S. review panel in Bethesda, Maryland, a researcher added a caveat: The inserted gene may have turned on growth signals, raising the potential for cancer.

So far this does not appear to be causing any harm, the study leader, Philippe Leboulch of the University of Paris and Harvard Medical School in Boston, told a meeting of the U.S. Recombinant DNA Advisory Committee (RAC). Leboulch's team in Paris received approval from a French regulatory review panel last month to treat more patients

for β -thalassemia. Still, the unexpected observation of a "clone," or large group of identical cells with the same gene insertion, suggests that the disabled HIV virus used to carry the new gene into the patient's cells may not live up to its billing as much safer than older types of vectors.

β -thalassemia involves a flaw in the gene coding for β -globin, one of the protein chains that make up the hemoglobin used by red blood cells to transport oxygen. The disease strikes mainly people of Mediterranean and Asian descent. The patient in this trial was typical: Since childhood, he has needed monthly blood transfusions and daily treatments to lower blood iron levels. He was not eligible for a bone marrow transplant, the only cure for the disease, according to Leboulch.

In June 2007, when the patient was 19 years old, Leboulch's team removed some of his bone marrow cells and treated them

with a modified HIV virus, or lentivirus, carrying a good copy of the β -globin gene. They infused the repaired cells into the patient; after several months, some of the cells began making β -globin. The patient's hemoglobin levels are now high enough that for the past 16 months he has not needed blood transfusions and feels well, says Leboulch.

A year after the treatment, however, Leboulch and his collaborators noticed something worrisome: A growing portion of the patient's modified blood cells contained an insertion in a gene called *HMGA2* that turned the gene on. This suggests that a single cell with this insertion gave rise to cells that had a growth advantage. The pattern is reminiscent of a gene therapy trial for an immune disease in which a different vector inserted near an oncogene, triggering leukemia.

That doesn't appear to be likely in the β -thalassemia case. Growth of *HMGA2*-

CREDIT: UKCMRI

the university's chemists, physicists, engineers, and other nonbiological scientists. As planned now, UCL will fund 5% to 10% of UKCMRI's cost, Wellcome Trust 20%, CRUK 25% to 30%, and MRC 45% to 50%.

Like many new science facilities, the designs for UKCMRI incorporate windows that allow the public a view into some laboratory space. To promote interaction across the whole facility, UKCMRI will not be broken up into traditional scientific departments but will have "neighborhoods," according to architect Larry Malcic of HOK London.

UKCMRI will not have its own patient facilities, although its primary goal remains ultimately translating biological insight into medical advances. UKCMRI officials confirmed that the facility will house an infectious disease facility allowing work on pathogens such as flu viruses but not on the most dangerous microbes such as Ebola. One-third of the proposed building will extend below the surface, including the animal facilities that will enable work only on small animals, including mice and likely ferrets, for flu studies.

The plans for UKCMRI haven't won over everyone. Jonathan Stoye, a virologist at NIMR, remains almost as concerned about the effort as he was in 2007, questioning whether the United Kingdom will get less science for its pound than it does now at NIMR. He notes that he and colleagues had to repeatedly argue for the inclusion of animal facilities at UKCMRI

and says, "I have very little faith in the current plan being the final plan."

Stoye sees other problems. Even though the sale of the NIMR and LRI sites should bring in huge sums, more money will be needed. So far, the Department of Treasury hasn't made a commitment to fill that gap. Nor has the local city council given approval for the project. With a national election due next year and expectations of budget cuts, UKCMRI proponents may have their work cut out for them: "They still have to make the business case to government, and it could be thrown out," notes Stoye.

Indeed, despite the drawing and blueprints presented this week, much remains uncertain. There's no clear explanation of how NIMR and LRI scientists will be selected for transfer to the new site. No one is guaranteed a spot; MRC chief Leszek Borysiewicz would say only that a "large fraction" of NIMR scientists would make the move. "It's a tricky issue," admits Treisman. "We've started to discuss how to discuss [the hiring process]."

No director of UKCMRI has been named yet. And there's no clear governance plan in place, although Nurse stresses that UKCMRI will be an independent institute and not run by MRC, UCL, or the charities. Smith ruefully acknowledges that "the name 'NIMR' will be no more"—at least not on U.K. soil. Smith recently had a star named after the institute to honor its legacy.

—JOHN TRAVIS

ScienceInsider

From the Science Policy Blog



India has created a **climate change assessment network** that links 220 scientists at 20 institutions. The goal, explained a government minister, is to develop an "independent and credible research capacity." It is expected to receive \$250 million over 5 years. <http://bit.ly/8rzoec>

In its second competition, the Department of Energy's **Advanced Research Projects Agency-Energy** has offered \$100 million for the best ideas in the fields of electro-fuels, carbon-capture technologies, and high-density battery storage. ARPA-E made 37 awards worth \$151 million in its first competition. <http://bit.ly/7Xaug5>

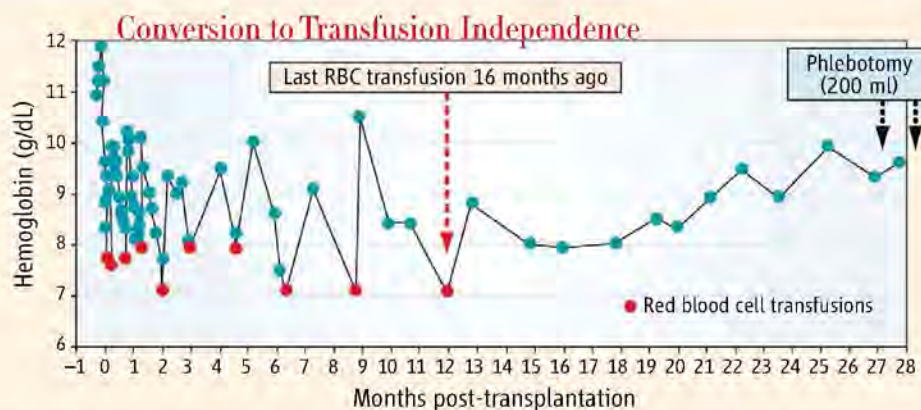
A researcher at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in Frederick, Maryland, contracted rabbit fever, or **tularemia**, and is responding well to medicine. It's a rarity for bioweapons scientists to become infected with the pathogens they study. <http://bit.ly/5Foc0C>

Long-simmering tensions between researchers and the business-oriented governing board at the **Australian synchrotron** in Melbourne erupted in an open battle as scientists went on strike to protest the firing of the lab's director, temporarily reducing the facility's normal 24-hour operation to a 9-to-5 schedule. <http://bit.ly/4FGngg>

A British official has confirmed that the government has recently resumed a controversial **pilot program to examine the DNA** and isotopes of tissue from asylum seekers, albeit with some changes. <http://bit.ly/5PjaUp>

White House budget officials are sticking with **small increases for science in the president's proposed 2011 budget**. That could mean only a 2.9% boost for the National Science Foundation, to \$7.2 billion, and a 1.6% bump for the Department of Energy's science shop, to \$5 billion. <http://bit.ly/8pHwox>

For the full postings and more, go to blogs.sciencemag.org/scienceinsider.



New blood. A year after gene therapy, a β -thalassemia patient no longer needed blood transfusions.

marked cells has leveled off at about 10% of the patient's blood cells. The insertion so far "has had no untoward effect," noted RAC member David Williams of Harvard and Children's Hospital Boston. He says the trial is "undoubtedly a success" and suggests that both thalassemia and a similar disorder, sickle cell disease, could be treated with gene therapy. Others, including RAC chair Howard Federoff of Georgetown University in Wash-

ington, D.C., say more evaluation is needed.

For gene therapists, the big question is whether U.S. regulators will approve the first U.S. trial for β -thalassemia. Michel Sadelain of Memorial Sloan-Kettering Cancer Center in New York City, who hopes to launch the trial by next spring, acknowledges that lentiviral vectors may not eliminate all risks: "Safer does not mean absolutely safe."

—JOCELYN KAISER

GENETICS

SNP Study Supports Southern Migration Route to Asia

A massive effort to catalog genetic variation among Asians has just weighed in on the peopling of that vast continent. As described on page 1541, a 40-institution consortium has concluded that Asia was initially settled by a single wave of migration along the coast; exactly when is still to be determined.

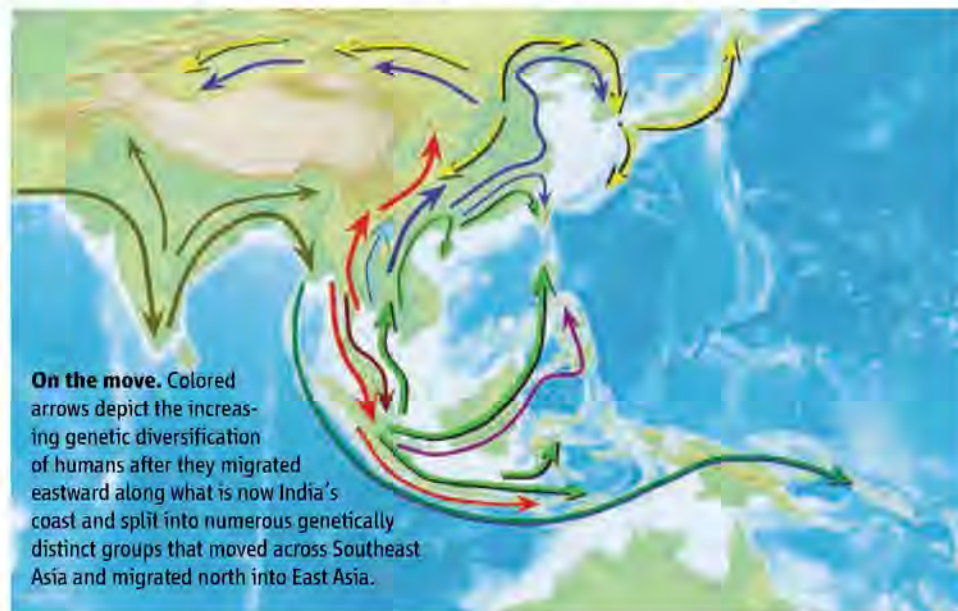
"It's a fabulous data set," says Vincent Macaulay, a statistical geneticist at the University of Glasgow in the United Kingdom. The evidence for the southern coastal route and against a northern steppe route "seems very strong," he adds.

Anthropologists, ethnographers, and linguists have long struggled to understand the patchwork-quilt diversity of Asia. Indonesia alone claims some 300 ethnic groups; the Philippines has 180 native languages and dialects. Where did they all come from? In recent years, geneticists joined in, using genomic markers to divine migration patterns and relationships among different ethnic groups.

These efforts produced two basic theories to explain the initial peopling of the continent. The dominant one pictures two major waves of migration from the Middle East. One wave followed a southern coastal route, around the rim of present-day India, and continued from island to island across Indonesia, Malaysia, and the Philippines to the Pacific; a separate and distinct wave of immigrants traveled east across the Eurasian steppe and turned south through the Asian mainland. A second theory posits just one initial migration—along the coastal route—with populations moving north into East Asia from there.

This new analysis by the HUGO Pan-Asian SNP Consortium "strongly concludes the southern route made a more important contribution to East and Southeast Asian populations than the northern route," says Li Jin, a population geneticist at Fudan University in Shanghai, China, and one of the paper's lead authors.

The results are significant for two other reasons. Scientifically, with samples from more than 1900 individuals representing 73 populations, "this is the most comprehensive study of genetic variation to date in East and Southeast Asia," says Noah Rosenberg, a bioinformaticist at the University of Michigan, Ann Arbor, who was not part of the consortium. Secondly, the consortium comprises 93 researchers at 40 institutions in 11 countries and regions in Asia, marking this a coming-of-age for the continent's genomic sciences. This project "was conceived by Asians in Asia and executed,



funded, and completed by an Asian consortium," adds Edison Liu, executive director of the Genome Institute of Singapore and one of the consortium's key organizers (*Science*, 3 December 2004, p. 1667).

Previous studies, mostly by researchers in Europe and North America, relied on limited numbers of samples from just a handful of Asia's ethnic populations. This local grassroots effort opened the door to larger numbers of more-representative samples.

The job wasn't always easy. About half of the 288 Indonesian samples had been collected previously, primarily from the country's major population groups. But tapping into subpopulations typically required numerous visits to village elders to explain the project, its objectives, and the concept of informed consent, says consortium organizer Sangkot Marzuki of the Eijkman Institute for Molecular Biology in Jakarta. One isolated group in central Sulawesi agreed to participate only if all of its 1000 members were given medical checkups. "So we went back with a mobile medical unit," Marzuki says.

Researchers screened each sample for more than 50,000 single-nucleotide polymorphisms (SNPs)—sites on chromosomes where a single base can vary from one individual to another. The number of variations, or haplotypes, indicates how closely related two individuals are genetically.

Not surprisingly, the genetic groupings strongly correlate with linguistic and geographic groupings. But the consortium also found that genetic diversity markedly

decreased going from south to north. In addition, most of the genetic variations found in East Asian populations were also present in the Southeast populations, indicating that the former likely derived from the latter. The authors conclude that humans migrated along a coastal route from the Mideast to Southeast Asia and from there moved north, gradually adapting to harsher climates.

Jin says their findings do not completely rule out a northern route migration. There is evidence of genetic links across the Eurasian steppe. But Jin thinks these links result from genes flowing back and forth along ancient trade routes rather than moving in as part of a large-scale migration. Macaulay says more data from central Asia could "hammer another hefty nail in the coffin of the [two-wave] model." Yet Rosenberg says the authors "leave some room for other multiple-wave theories," specifically that several different migrations across the southern route could account for the distinct populations seen in New Guinea, Australia, and the Pacific Islands.

The consortium intends to address these questions in a second project that Liu says will be more ambitious geographically—including, the consortium hopes, central Asian and Pacific Island nations—and scientifically, analyzing far more genetic markers to map diversity and to extend the work to genomic medicine. With the experience gained on the consortium's first project, the riddles of Asia's diversity may yet be solved by the unified efforts of Asian scientists.

—DENNIS NORMILE

CREDIT: L. JIN ET AL., SCIENCE

CHINA

Internet Blockade in Xinjiang Puts A Strain on Science

URUMQI, CHINA—Few researchers can go a day without checking e-mail. Imagine being deprived of Internet access—and being unable to send text messages or make international calls—for 5 months and counting. Such an unprecedented disruption is now hobbling the daily routines of thousands of scientists and students here in Xinjiang Uyghur Autonomous Region. The telecom blockade has affected a U.S.-supported HIV-prevention trial and could jeopardize an effort to build a research base in China's vast western frontier. "If this situation continues, it will affect the region's development," says Chen Yiyu, president of the National Natural Science Foundation of China (NSFC).

The Chinese government imposed the communications blackout (domestic phone service is unaffected) on 6 July, 24 hours after a protest by Uyghurs here in Xinjiang's capital devolved into riots in which scores of people, primarily Han Chinese, were killed. Authorities have asserted that Uyghur separatists in Xinjiang and abroad used social networking Web sites like Facebook and Twitter to orchestrate the violence.

In the wake of the unrest, machine gun-toting soldiers and militias have kept the peace. Ecologist Yang Weikang of the Xinjiang Institute of Ecology and Geography (XIEG) of the Chinese Academy of Sciences (CAS) says that in the days following the mob violence he wouldn't have risked leaving his home at night. Now, he says, "we no longer feel unsafe to be out on the streets." But the Internet remains off limits to the public, with the exception of some officials and journalists allowed access at a downtown site. "This has had a big impact on our work," says XIEG's Liu Wenjiang.

One international project disrupted by the blockade is a novel trial testing whether the antiaddiction drug Suboxone can stem HIV infections by treating heroin addiction. Injecting drug use is a leading route of HIV transmission in western China. Last year, a team of Chinese and U.S. scientists led by prevention researcher David Metzger of the University of Pennsylvania launched the 4-year project funded by the U.S. National Institutes of Health in Ürümqi and Heng County in China's Guangxi Zhuang Autonomous Region. With international links to Xinjiang severed, messages and data are

being passed to and from Ürümqi via Guangxi. "The inability to interact directly with the staff is a serious concern," says Metzger.

Cyberisolation is forcing scholars elsewhere in Xinjiang to find alternative ways to conduct research and communicate with peers. Researchers at Ürümqi Astronomical Observatory at Nanshan are unable to download daily reports from other observatories; it takes 3 to 4 days to receive reports by express mail from Shanghai. The observatory's deputy director, astronomer Sun Zhengwen, dispatches staff and students on regular trips beyond Xinjiang to commune with colleagues and prepare papers for submission to journals. But because Sun is in charge of the observatory's security, he must stay put. "It's frustrating," he says. Likewise, XIEG scientists are beating a path to Beijing. Meetings in Ürümqi have been postponed, and a CAS-U.S. National Science Foundation workshop last month on the ecology of the Tarim Basin was moved to Xi'an, says Liu.

Scholars who cannot easily travel outside Xinjiang—

net outage could be severe. This year, NSFC is spending \$30 million on more than 600 projects in Xinjiang and four other western provinces to beef up the region's frail science base. "The idea is to keep excellent scientists in the local institutes," says Chen. Funding decisions were largely wrapped up before the riots, but Chen worries about next year's call for proposals. If the situation drags on, he says, Xinjiang's woes could spark an exodus of scientific talent.

Some academics have managed to find a silver lining in the info blackout. He Jing, an English instructor at Xinjiang University, told the state-owned newspaper *China Daily* that lack of Internet access has cut down on



Anybody out there? An Internet outage imposed after July's riots in Ürümqi has slowed communications at the Ürümqi Astronomical Observatory and other sites.



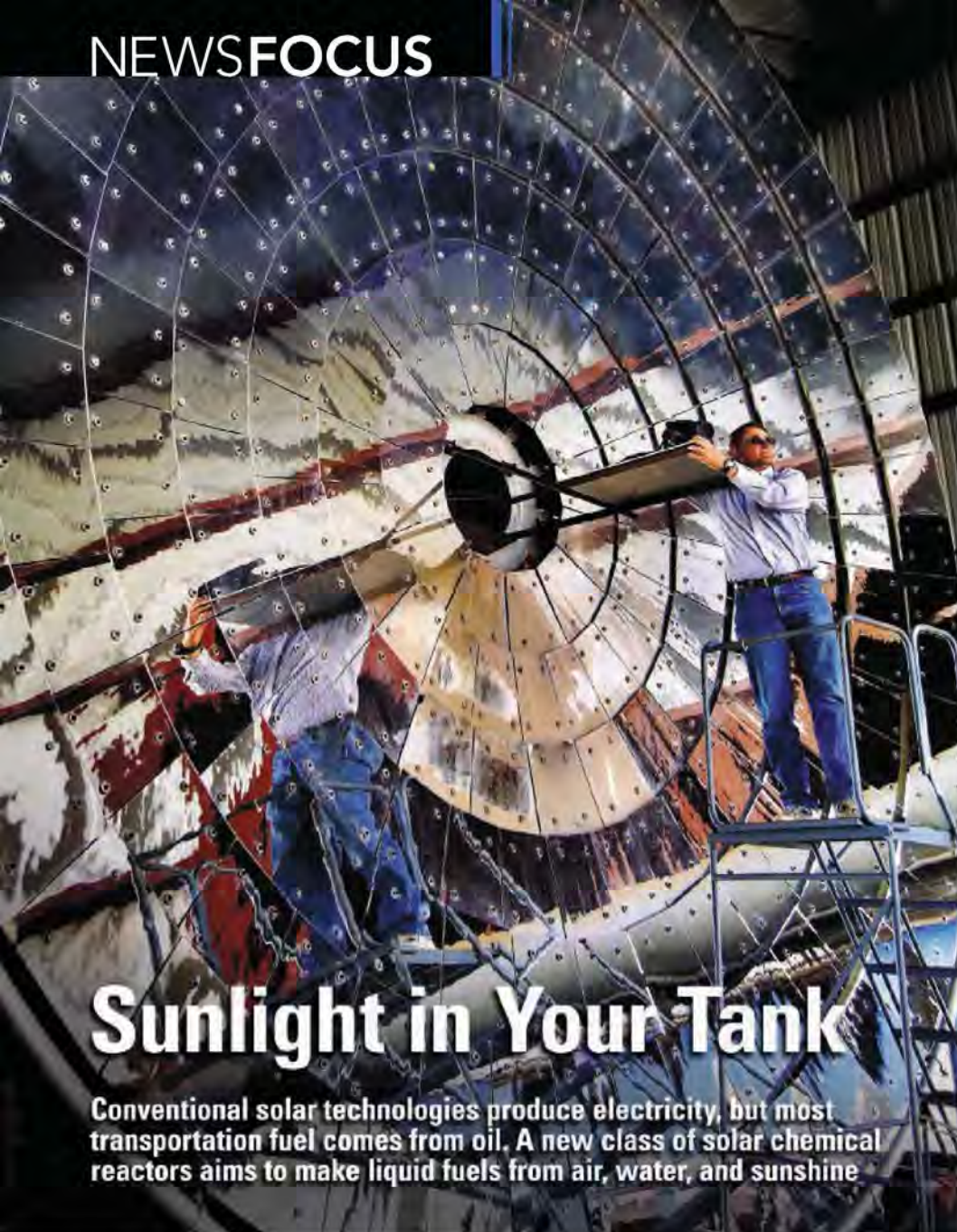
plagiarism among his students, who often copy material verbatim from the Web. "Now they have to do everything on their own," he said. But the vast majority of researchers are anxious to come in from the cold.

Authorities are mum on when Xinjiang might be back online. In late summer, rumors predicted service would be restored after the National Day festivities on 1 October. But as the outage approaches a half-year, researchers here have resigned themselves to finding novel ways to reach out to the world and maintain the rhythm of their scientific lives.

—RICHARD STONE

students or academics with fewer resources or those who conduct fieldwork—must find other lifelines, such as colleagues in Beijing who can check their e-mail and relay important messages by phone. To ease the scholars' plight, CAS headquarters has set up a "Green Channel" through which Xinjiang researchers can call and request articles for express mail delivery.

The consequences of an indefinite Inter-



Sunlight in Your Tank

Conventional solar technologies produce electricity, but most transportation fuel comes from oil. A new class of solar chemical reactors aims to make liquid fuels from air, water, and sunshine

ALBUQUERQUE, NEW MEXICO—Inventors seem to have a thing for garages. So it's fitting that Rich Diver's workplace looks like an oversized carport, a metal shed some 8 meters high and 8 meters across sitting under the desert sun. Diver, a solar engineer here at Sandia National Laboratories, flicks a switch, raising the shed's oversized door. Just outside sits a heliostat, a large, flat mirror that tracks the sun, and what looks like a huge window frame holding a massive venetian blind. Inside the shed, propped up on its side, rests a 6.5-meter-wide solar concentrator, a parabolic dish covered with 228 mirrors. During operation, sunlight ricochets off the heliostat, passes through the venetian blind, and hits the parabolic dish, which focuses it on a small keg-sized chemical reactor. Temperatures at the focal point can reach more than 1500°C, hot enough to burn through a

fire brick used to line kilns. Diver and his colleagues are using the heat and an iron oxide catalyst in the reactor to split either water or carbon dioxide (CO₂), producing hydrogen gas or carbon monoxide. These chemicals can then be used as energy-rich feedstocks for making liquid fuels such as gasoline. In short, the Sandia team is reversing the combustion of fossil fuels, reenergizing molecules from thin air (or water) to make fuels that can then be used anytime, anywhere.

Just not quite yet. At the moment, Diver's reactor is in pieces all around the garage. In the dust covering one of the mirrors is scrawled "I ♥ solar" and "Hi Dad," written by Diver's daughter Tracy during a recent visit.

Online
sciencemag.org

S Podcast interview
with author
Robert F. Service.

Concentrate. Sandia's Richard Diver works on a parabolic dish that focuses sunlight on a chemical reactor.

Diver disassembled the reactor after a recent test run that showed that iron oxide catalysts worked efficiently to split water. But the temperatures got so high, and the temperature changes so dramatic, that they fractured many of the flat, 1-cm-square catalyst tiles and cracked the ceramic housing holding them in place.

Back in town at the Albuquerque Marriott, Diver and about 30 of his colleagues from around the world have spent most of the past 2 days huddled in conference rooms searching for solutions to these sorts of problems and comparing notes on different versions of the technology.* They've sorted through whiteboards full of technical challenges with their solar reactors, ranging from boosting the speed at which the catalysts work to keeping the windows on the reactors clean so the focused sunlight can stream in.

The good news is that the technology has been shown to work on a small scale, producing tens to hundreds of liters of energy-rich gases per hour. "This is not a pipe dream," says Alan Weimer, a chemical engineer and solar-fuels expert at the University of Colorado, Boulder. "We have things that actually work." And a simpler version of the technology is already close to market (see sidebar, p. 1474).

But there's bad news, too. For now, the process is costly and unlikely ever to compete with producing gasoline from fossil fuels without a price on carbon emissions. "We are not at the point where it is feasible on a commercial scale," Weimer says. Diver agrees: "It puts us in this in-between land." This awkward stage of development is a crowded territory known as the "valley of death" among venture capitalists, who specialize in turning promising technologies into moneymaking businesses. Diver and his colleagues have assembled here in hopes of finding a way out of this valley. Their immediate goal has been to put together a white paper that lays out the case for solar fuels—more officially known as solar thermochemical fuels, because heat from concentrated sunlight drives the needed chemical reactions.

This search for the way out of the valley of death has become the quintessential problem facing energy researchers of all stripes today. Whether they work in a relatively sparsely populated area such as solar fuels or in cellulosic ethanol, which is widely hailed to be the

*Workshop on Solar Thermochemical Cycles, 2–4 November 2009, Albuquerque, New Mexico.

future of ethanol technology, they must develop a new technology for producing fuel or electricity. And they must do it cheaply enough to compete with fossil fuels, a technology that engineers have been whipping into shape for more than 100 years and that has trillions of dollars of infrastructure installed to make it work.

At a break during the meeting here, Diver is chatting with Robert Palumbo, a mechanical engineer at Valparaiso University in Indiana. "Is what you are doing harder or easier than landing a man on the moon?" Palumbo asks. "Harder," Diver says without hesitation. "They only had to do it once." Besides, he adds, "they didn't have to worry about economics."

A matter of scale

NASA engineers were also the beneficiaries of a sustained financial push to develop the technology. Not so with solar thermochemical fuels. The field got an initial boost after the Arab Oil Embargo of the early 1970s raised concerns about rising oil prices and Western countries' dependence on Middle Eastern oil. Groups in Europe, the United States, and Japan spent a few years searching for catalysts to convert heat into fuels regardless of the heat source, but interest had largely died out by 1983 as oil prices again plunged. Solar-fuels programs in Switzerland and Germany took off again in the early 1990s, driven by growing concerns over climate change and fears that world oil production was nearing its peak. Beginning in 2003, a brief bolus of funding for producing hydrogen fuel kick-started several efforts in the United States, including the Sandia effort, but that funding has since disappeared.

Despite the ebbs and flows of funding, attendees at the Albuquerque meeting argue that the need for solar-fuels research is only growing. Anxiety about rising atmospheric CO₂ levels—now at 385 parts per million, up 43% from preindustrial levels—"is the underlying premise of why we are here," says James Miller, a materials scientist at Sandia. Miller helps run the lab's Sunshine to Petrol program, which is now supported by internal Sandia funding. Energy security is another concern. With 58% of U.S. oil now coming from imports, Miller notes, "the transportation sector is very vulnerable to disruptions in supply."

But a potential plus for solar thermochemical fuels is one that renewable-energy providers rarely mention: scalability. Energy use occurs on a scale unlike any other. In 2006, for example, humans used 472 quadrillion BTUs (quads) of energy, 21% of it in the United States. More than ¼ of



Re-energize. Solar engineer Richard Diver of Sandia helped develop this reactor, which uses heat from concentrated sunlight to split water into hydrogen and oxygen, and carbon dioxide into carbon monoxide and oxygen.

the U.S. portion went to transportation, and nearly all of that came from oil. As a result, any new technology hoping to dethrone oil drilling will have to supply not only cheap energy but a lot of it. That's where most renewables hit the skids.

Take biofuels. In 2007, Congress mandated that by 2022 the United States will make an estimated 117 billion liters of ethanol. Producing it is expected to require anywhere from tens of millions to hundreds of millions of hectares of land, depending on future efficiency increases in turning biomass into ethanol. Even so, the 2022 ethanol supply is expected to meet only about 13% of U.S. demand for transportation fuel. With biofuels already increasing the pressure on agricultural and conservation reserve lands, it's hard to imagine the figure approaching 100%.

That's where concentrated solar-fuel plants have an important advantage, says Ellen Stechel, who heads Sandia's Fuels and Energy Transitions Department. Because the technology uses the full solar spectrum and concentrates sunlight, it uses far less land than biomass, photovoltaics, and other technologies that also rely on capturing sunlight. That could make it far more straightforward for the technology someday to supply a large fraction of transportation fuels.

At the Albuquerque meeting, Robert Wegeng, a mechanical engineer at the Pacific Northwest National Laboratory in Richland, Washington, walked attendees through an initial calculation that shows the potential of an intermediate, and simpler, version of solar-fuel technology. Wegeng is developing a solar reactor capable of converting natural gas to a mixture of hydrogen and carbon monoxide—

known as synthesis gas—that can be used to make liquid fuels.

Wegeng calculates that a solar-fuel plant made up of an array of 10,000 parabolic dishes would take up only a few square kilometers of land. Yet it could capture 1 gigawatt of energy, enough to upgrade natural gas equivalent to 2 million gallons of gasoline per day or 700 million gallons per year. "Twenty such plants would offset U.S. petroleum exports by 10%," Wegeng says. And there's plenty of desert land to scale the technology up even further.

"This can potentially make as much fuel as we want," Stechel says. Even better, because the technology can be used as the first step to producing liquid hydrocarbons, it would allow countries to continue to use their built-up gasoline-based infrastructure without having to rely on mining fossil fuels.

Wegeng and others at the Albuquerque meeting were quick to add that they don't think solar fuels should be considered as an alternative to biofuels. "There is no silver bullet when it comes to replacing fossil fuels," Palumbo says. Instead, solar thermochemical fuels are one of the pieces of "silver buckshot" needed to do the job. Stechel agrees. "It's another good option," she says. "We need them all."

Next hurdle: Efficiency

In reality, solar thermal fuel technologies are several pieces of buckshot, racing toward the same target: the simple ability to split water or CO₂. These compounds can be split directly with heat, but they can create an explosive mixture of gases that need to be separated. By using catalysts, researchers



Biomass Fuel Starts to See the Light

Even with a major push, commercial plants capable of turning CO₂ or water into liquid fuels are still likely to be 2 decades away. A simpler version of the technology, however, already appears headed to market. Sundrop Fuels Inc., an energy start-up based in Louisville, Colorado, recently commissioned a 1-megawatt solar array to convert wood waste and other forms of biomass into a gaseous blend of carbon monoxide and hydrogen—known as synthesis gas—that can be converted into gasoline. The plant uses an array of 2700 mirrors to concentrate sunlight on a 20-meter-tall solar tower to produce heat needed to drive the chemical reactor. The company has told *Science* that in 2012 it intends to open a commercial plant capable of capturing 60 megawatts and use that energy to produce 19 million liters of gasoline annually.

"This is a very good development," says James Miller, a materials scientist at Sandia National Laboratories in Albuquerque, New Mexico, who helps direct the lab's Sunshine to Petrol program. "If this is successful, it will help demonstrate that solar thermal energy will be an important piece of how we produce liquid transportation fuels in the future."

At a recent workshop on solar fuels, participants spent considerable time debating whether the small solar-fuels research community should push for large-scale demonstration plants akin to Sundrop's. Such plants offer a way to take a carbon source, such as biomass or coal, and increase its energy content while converting it into a liquid hydrocarbon. "On the one hand, it's a distraction" from the ultimate goal of converting CO₂ to fuel with sunlight, says Ellen Stechel, who heads Sandia's Fuels and Energy Transitions Department. "But on the other hand, it's a steppingstone to where we want to be."

That steppingstone is easier to reach because it employs a simpler chemical reactor. Splitting CO₂ and water requires specialized catalysts that must be transferred between two separate stages to carry out different reactions. Converting biomass to syngas, by contrast, requires only quickly heating the biomass in the presence of steam. Biomass gasification in conventional chemical reactors has been explored for decades. But the technology has been held back in part because some of the biomass itself, or other fossil fuels, must be burned for heat to power the reaction. Getting the heat from solar concentrators eliminates that heating cost and avoids generating excess CO₂. But how the initial cost of the solar concentrators affects the economics of the process remains to be seen. "Building a demonstration plant gives you the opportunity to work some of those issues out," Miller says.

In related work, researchers around the globe are investigating using heat from concentrated solar energy to convert methane in natural gas into liquid fuels, turn limestone into cement, and reduce metal oxides into metals. These industrial processes currently use massive amounts of energy and emit copious quantities of CO₂.

Jane Davidson, a mechanical engineer at the University of Minnesota, Minneapolis, says solar thermal systems are ready to take this first big step. "This is realistic," Davidson says. "This can happen, and it can make a big difference."

—R.F.S.

can reduce the temperature of their reactors and produce H₂, CO, and O₂ in separate steps, eliminating the separation problem.

Take Diver's setup, at least when it's not scattered in pieces around the garage. Inside the reactor sit 14 cobalt ferrite rings, made from a mixture of cobalt and iron oxide, or rust. Each ring is about 1/3 of a meter in diameter and rotates between two separate reaction chambers. Sunlight from the heliostat and the parabolic mirror is focused through a window in the reactor, heating the interior to as high as

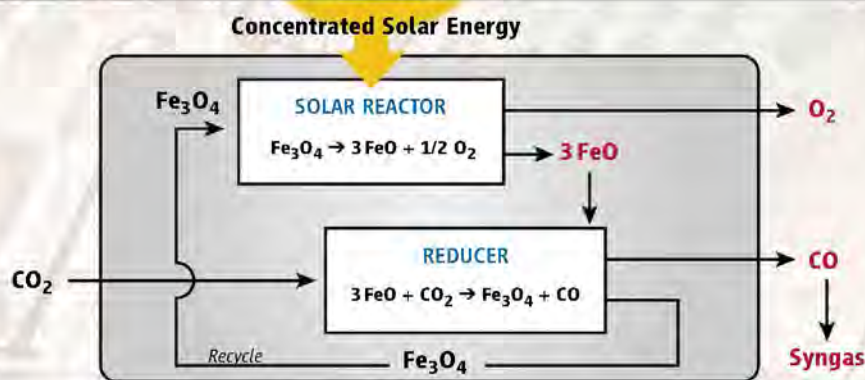
1500°C. At that temperature, the flakes of iron oxide catalyst on the outside of each ring spit out oxygen molecules, which are vented to the outside (see figure, below). As the disks turn, they enter the second, 1100°C chamber, which is spiked with CO₂ or water vapor from which the catalyst swipes oxygen atoms. This reaction generates the energetic gases (H₂ or CO), which are collected, and restores the catalyst to its original composition, ready for another turn of the wheel.

Other reactors use different catalysts and

in some cases different designs. A group led by Aldo Steinfeld, who heads the Solar Technology Laboratory at the Paul Scherrer Institute in Villigen, Switzerland, cycles a catalyst between zinc oxide and metallic zinc. The process has already proved to convert 3.1% of incoming solar thermal energy into chemical energy. Researchers at the German Aerospace Center in Cologne and Almeria, Spain, use their own blend of iron oxide-based catalysts. They said at the meeting that they are seeing efficiency numbers similar to those of Steinfeld's group. Finally, groups at the California Institute of Technology and the University of Minnesota have been experimenting with a promising catalyst based on the metal ceria, and both the Sandia and Paul Scherrer teams are now gearing up to test ceria catalysts in their reactors.

For now, however, none of these catalysts is good enough. Although the iron oxides can give up a large percentage of their oxygen atoms in the first, high-temperature step, they melt at 1800°C, just above the typical temperature in the reactors. And when they cool, they form an inert slag that is far less reactive in subsequent catalytic cycles. The iron oxide portion is also brittle and unstable. So it is typically mixed with large quantities of inert

Fe₃O₄/3FeO Thermochemical Cycle



Round and round. In this reactor, heat from concentrated sunlight converts a magnetite (Fe₃O₄) catalyst into wüstite (FeO) and oxygen. A second step swipes oxygen to restore the catalyst.



Mining the sun. This solar thermochemical fuels setup in Switzerland focuses sunlight on a reactor (*right*) that splits either water or CO₂ with the help of a zinc-based catalyst.

ceramics, which must be heated even though they don't perform any catalytic work. Moreover, because the ferrites are relatively large chunks of solid material, not all of the catalytic material in the center of each flake participates in the reaction.

Zinc oxide, by contrast, dissociates in a 1700°C reactor to form a zinc vapor and oxygen, thus ensuring that nearly all of it reacts. But the zinc vapor can condense on the reactor's window, blocking the incoming sunlight. Ceria is solid like the ferrites and is quick to take up and release oxygen. But because it grabs and releases less oxygen than the ferrites do, a reactor must include more of it. Right now, Stechel says, "there are lots of different tradeoffs." However, she adds, "we have not even begun to explore the full material spectrum" of possible catalysts out there.

For now, the Swiss group has the best reported efficiency in converting heat from incoming sunlight into fuel, at just over 3%. For the technology to have a shot commercially, however, solar-fuels researchers say they will need to do significantly better. Solar electric generators are already 20% efficient at converting sunlight to electricity. And current commercial electrolyzers can convert electrical energy to hydrogen gas at 65% efficiency. By combining the two devices, engineers can convert sunlight into hydrogen at more than 13% efficiency with conventional technology. "We're trying to do it more

directly and more efficiently," Miller says.

There's reason for optimism, Miller adds: The theoretical maximum efficiency of converting heat to chemical bonds is 75%, limited by the Carnot efficiency of the heat engine. For a successful catalyst, he says, "we only have to get to about half of that," probably resulting in an overall reactor efficiency of slightly more than 20%. "A factor of less than 10 improvement certainly seems manageable," Miller concludes.

Others agree and suggest that the goal for the field should be a reactor that converts 20% of incoming heat to fuel by 2020. Better efficiency will have other payoffs as well. Most important, it could enable engineers to use smaller concentrators and heliostats, which account for roughly half the cost of current solar thermal systems.

So how much would fuel from concentrated sunlight likely cost? "We are confident we can get this number down to less than \$10 for a gallon of gas," Miller says. At that price, Steinfeld says, "it's clear solar fuels will have a hard time competing directly with fossil fuels." But if the world begins to move away from fossil fuels to CO₂-neutral fuels, Steinfeld says he's convinced that solar thermochemical reactors



could step in to meet the demand.

Improving catalyst efficiency and getting the price down won't be the only hurdles solar-fuels researchers have to leap. Catalyst lifetimes in the reactors need to improve. And researchers will need to find a way to run the reactors on stored heat at night so that future commercial plants can run 24/7, typically an industry and financial requirement. But even though that list sounds daunting, solar-fuel researchers insist that most of the items are fairly standard engineering challenges that will be overcome with sustained work on the technology. "There is every reason to believe there are no showstoppers here and it will be viable," Stechel says. The question now, and the hardest challenge ahead, is whether the field's successes to date and promise for the future will persuade funding agencies to put them on the path to commercial reality.

—ROBERT F. SERVICE

AIDS VACCINE RESEARCH

HIV Natural Resistance Field Finally Overcomes Resistance

People who fend off HIV despite repeated exposures may have genetic or immunologic factors working for them that can help guide AIDS vaccine research

WINNIPEG, CANADA—In 1989, Stuart Shapiro and his pregnant wife, Awuor, went to the obstetrician to learn whether she carried the gene for sickle cell anemia common in people from Kenya, her home country. The results shocked the young couple: No, she did not have the gene, but the doctor said Awuor was infected with HIV. “With one sentence, our world fell apart,” said Shapiro, who had been with Awuor for 7 years. The doctor suggested that they consider terminating the pregnancy, but Awuor carried to term and gave birth to a daughter.

To their great relief, Stuart was not infected, and Awuor did not transmit the virus to their daughter. In part because he wanted inside information about the latest anti-HIV drugs to help Awuor, Stuart, a retrovirologist, went to work for the U.S. Food and Drug Administration. But Awuor’s health steadily deteriorated, and in 1996, she died from AIDS. As the years passed, Stuart became increasingly convinced that studies of his blood might help spare others from the fate his wife had suffered. He had, after all, likely been exposed to the virus repeatedly. If some combination of genetic and immunologic factors had protected him, unraveling them might inform researchers struggling to develop an AIDS vaccine and other biomedical preventives.

From 15 to 17 November, the first-ever meeting on natural immunity to HIV was held in this small city on the Canadian prairie, and Shapiro was one of 100 scientists from around the world who attended. Now a program officer at the U.S. National Institute of Allergy and Infectious Diseases (NIAID), Shapiro oversees a \$50 million annual grant to an academic consortium called the Center for HIV/AIDS Vaccine Immunology (CHAVI). At the meeting, he not only represented NIAID but also spoke for other people who have dodged the HIV bullet and donated their blood for study, and who are frustrated that the field remains so dis-

jointed and underappreciated. “I want them to study the phenomenon extensively,” said Shapiro. “I don’t want them to leave any stone unturned.”

Just as Edward Jenner used milkmaids who did not develop smallpox to design a vaccine that ultimately eradicated that disease, researchers here had high hopes that their findings will help in designing an effective AIDS vaccine. But so far, few tangible advances have come from studying people like Shapiro, despite years of efforts.

Dozens of studies have been examining men and women who sell sex but remain uninfected, and similarly, clients of sex workers, “discordant couples” like Shapiro and his wife, hemophiliacs who received tainted lots of blood, babies like Shapiro’s daughter, injecting drug users, health care workers who accidentally poked themselves with contaminated needles, and men who have sex with many male partners. But all too often the leads point in contradictory directions, in part because investigators use different assays to probe their samples, and there is little coordination among them. Many labs also use wildly varying criteria to decide who qualifies as HIV-resistant, making it difficult to sort out which study subjects were truly exposed and uninfected, were exposed and have an occult infection, or were never exposed in the first place. “If we are getting anything out of this meeting, it will be

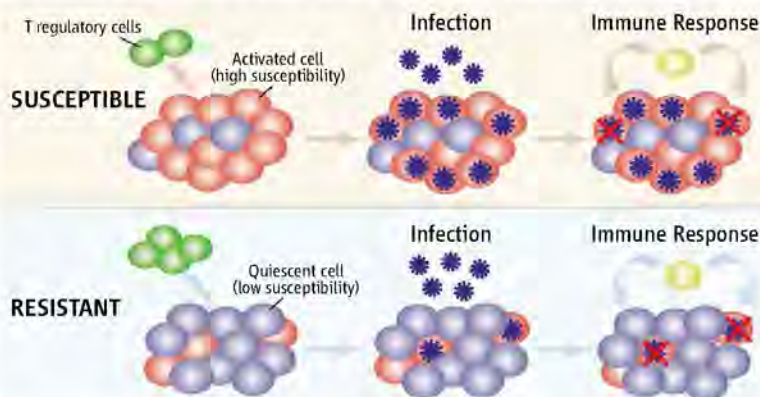


Healthy relationship. Stuart and Akinyi Shapiro lost a wife and mother to AIDS, but neither became infected. Why?

to come to some agreement about the people we’re studying and the degree of risk they have faced,” said Frank Plummer, the meeting’s organizer and head of Canada’s National Microbiology Laboratory, which is based here and has an \$8.3 million grant from the Bill and Melinda Gates Foundation to study natural immunity. “And it’s quite possible there are different mechanisms at work in different groups.”

For more than 20 years, Plummer has followed a group of sex workers in a Nairobi slum that includes more than 100 women who by all odds should have become infected but are not. With a nudge from the Gates Foundation, Plummer and his team invited colleagues who have similar cohorts to Winnipeg to see whether they could all collaborate. “Before, this was seen as a freakish phenomenon,” said Mario Clerici, an immunologist at the University of Milan in Italy who helped pioneer the field with Gene Shearer of the U.S. National Cancer Institute (NCI). “Gene and I were totally in left field: Nobody believed natural immunity to HIV existed. Now opinion leaders are jumping into it.”

Studies of the Nairobi sex workers continue to provide clues about protection. Keith Fowke, a microbiologist at the University of Manitoba here



Quiet type. People resistant to HIV may have fewer “activated” cells, presenting the virus fewer targets and making it easier to clear the few infections that do occur.

CREDITS (TOP TO BOTTOM): PHOTO BY STACY ZARIN GOLDBERG; (SOURCE) KEITH FOWKE/UNIVERSITY OF MANITOBA

who works with Plummer, explained that they have focused on the 5% of more than 3000 women in their cohort who on sensitive PCR blood tests remain uninfected by HIV after at least 3 years of selling sex. Some of the women deemed resistant have later become infected, leading Fowke to stress that the protection against HIV is not absolute. Yet he is certain that these resistant women have genetic and immune responses that helped them thwart the virus. Fowke, Plummer, and their co-workers at the University of Nairobi have homed in on the resistant sex workers' unusual CD4 white blood cells, which coordinate the immune response against HIV and are also its main target. Unlike people at low risk of HIV infections, CD4 cells from these women copy themselves vigorously—or "activate"—when exposed to pieces of the virus in test tube studies, indicating that they have confronted the enemy before and can quickly rev up the immune system to block infection. But these HIV-specific first responders make up a tiny fraction of the CD4 cell population. And the general CD4 cell population in the resistant women had unusually low levels of activation markers. This indicates that most of their CD4 cells are in a "resting," or quiescent, state, save for those that are actively responding to HIV—and that may be key to their resistance.

Various cellular factors make it difficult for HIV to infect resting CD4 cells in the blood, forcing the virus to rely mostly on the activated ones to establish an infection. So smaller populations of activated CD4s mean fewer targets for HIV. In something of a paradox, activation is good when it's directed against HIV but bad when CD4s are turned on high for other reasons.

In support of their immune-quiescence hypothesis, Plummer, Fowke, and colleagues found that the resistant women had higher levels of regulatory T cells that directly tamp down activation. When Fowke's lab compared gene expression in CD4 cells in resistant women and uninfected controls, the resistant group had far fewer genes turned on high, also suggesting a quiescent state.

Fowke suspects that the reduced number of targets in immunologically quiescent people makes it harder for HIV to succeed because even if a few cells do become infected, the immune system has a better chance of snuffing out the fire before it spreads. It made a compelling story, but as often happens in this field, Clerici followed with a study in discordant heterosexuals in Milan that suggested activation *helped* people avoid HIV, putting a damper on the quiescence theory. "I have data that are absolutely the opposite," apologized

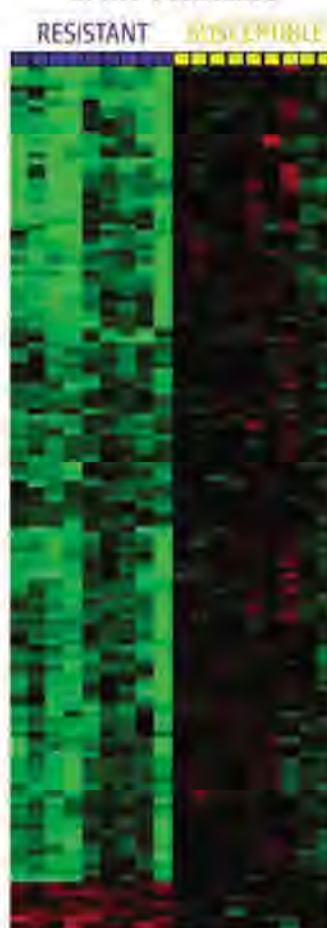
Clerici, who noted differences in the way they analyzed samples. "I don't know what to say."

Other disparate findings presented here suggest that myriad factors contribute to HIV resistance. Activation is part of the refined adaptive immune system that remembers specific pathogens and mounts attacks when they reappear. Gianfranco Pancino of the Pasteur Institute in Paris linked protection in exposed injecting drug users in Vietnam to the more primitive innate immune system, showing that they had unusually high levels of natural killer cells—which are much less selective than adaptive immune actors like HIV-specific CD4s—to clear infections. Kristina Broliden and Klara Hasselrot of the Karolinska University Hospital in Stockholm found that uninfected men who had oral sex with long-term infected male partners developed powerful "neutralizing" HIV antibodies in saliva that might prevent infection by that route. Several genetic studies identified a handful of specific genes associated with protection that may turn on underappreciated immune responses.

Immunologist Michael Lederman of Case Western Reserve University in Cleveland, Ohio, found the often-conflicting leads sobering. "This is one of the most interesting and important problems that's facing HIV research today," he said. "But I'm actually quite pessimistic that we're going to be able to sort this out."

Lederman said he was not confident that the populations being studied were "clean enough" to fish out correlates of protection. "When we're talking about folks who are exposed through sexual exposure, quantifying the risk is kind of difficult," he said, because there is no way to determine what HIV dose—if any—they have actually thwarted. So Lederman has turned to studying the carefully characterized groups of uninfected hemophiliacs who received contaminated lots of blood-clotting factors in the early 1980s. As

Gene Expression Profiling In HIV Resistance



Gene Expression Levels
Inside CD4 T Cells

Note:
Columns = individuals;
Rows = genes

Array of hope? Genes inside the immune cells of HIV-exposed, resistant sex workers in Kenya are not expressed as much (green) as those in susceptible, uninfected people.

a group led by NCI's James Goedert showed in 1994, just 5% of hemophiliacs in the United States and Europe who received moderate to high doses of contaminated clotting factor did not become infected. That makes this group of uninfected hemophiliacs the most unequivocally resistant people to study.

Lederman and co-workers compared stored blood samples of 36 HIV-uninfected hemophiliacs from this group with samples from healthy controls. They looked for differences in susceptibility to infection, neutralizing antibodies, and chemokines but found nothing notable. The team did discover, however, that their cells were less likely to be activated to copy themselves, supporting the Plummer group's findings. But Lederman remained circumspect: "Correlation does not confirm causality."

Investigators working with CHAVI recently began intensive genetic and immunologic studies of blood samples from additional hemophiliacs who meet the same criteria. Shapiro says they hope to have 600 samples within the year, and they have already started to do genome-wide association studies and full-

genome sequencing of some individuals. CHAVI is also ramping up resistance studies in men who have sex with men, discordant couples, and mother-infant pairs.

By meeting's end, the researchers agreed to form a consortium to hammer out how they will share samples, standardize assays, and agree on definitions of HIV resistance. Although sorting wheat from chaff will remain challenging, one thing will not, says Shapiro: finding HIV-resistant people to volunteer for the studies. "We all wonder why we didn't get infected," said Shapiro. "It's almost like being a Holocaust survivor. And if studying us can help bring an end to the epidemic, it can help us make some sense of our lives and the suffering we've seen and felt."

—JON COHEN



HUMAN EVOLUTION

What's for Dinner? Researchers Seek Our Ancestors' Answers

To help prevent diseases like diabetes and heart disease today, evolutionary biologists seek to understand the tastes of our diverse ancestors

BERLIN—As evolutionary scientists from around the world loaded their plates with fish, potatoes, and pork at a lunch buffet at the Berlin Medical Historical Museum, talk naturally turned to what's best for humans to eat. Compared with us, our ancestors ate "meat. More protein, less refined carbohydrates, and no milk," pronounced exercise physiologist Loren Cordain of Colorado State University, Fort Collins, who advocates a similar regimen to prevent disease. (He passed up the pasta.) At a nearby table, though, his colleagues ate ... pudding.

Sixteen researchers from multiple disciplines chewed on the question of whether there is an ideal diet for humans as part of a recent workshop on evolution and modern diseases.* Those focusing on diet hoped to test the common belief that diseases such as obesity, diabetes, and high blood pressure arise because our bodies are poorly adapted to the modern diet, rich in fat, sugar, and salt. "Is there a single Paleolithic diet that is going to be a magic bullet for everyone?" asked biological anthropologist William Leonard of Northwestern University in Evanston, Illinois, who led the diet work-

shop. "Or do we have to tailor diets specifically to regional populations?"

After comparing emerging evidence from ancient humans and diverse modern cultures, the researchers concluded that many factors—including genes, sex, ancestry, and fetal and childhood conditions—influence how we digest foods and store fat. Physiological stress in mothers can leave lingering imprints on descendants for generations. So although it's true that humans evolved to eat a diet relatively high in protein and low in carbohydrates and fat, there's no single Paleolithic prescription for better health. "It is the internal environment inside yourself that is key—how genes are expressed and how you started off in life," says paleoanthropologist Peter Ungar of the University of Arkansas, Fayetteville.

The first suppers

From the beginning, our ancestors had varied tastes. "Early humans had many choices as they bellied up to the biospheric buffet," says Ungar. The 3- to 4-million-year-old australopithecines were omnivores who ate a wider range of foods than chimpanzees or other apes, according to microscopic wear patterns on fossil teeth and chemical isotopes in tooth enamel. Soon after the origin of our genus *Homo* about 2 million years ago, our lineage

Daily grind. The Tsimane' people in lowland Bolivia work hard for their food and have thrifty bodies that can survive on fewer calories.

began to eat more meat, butchering it with stone tools (*Science*, 15 June 2007, p. 1558).

By the time modern humans swept into Europe about 40,000 years ago, these hunter-gatherers were adept at hunting large game and had also expanded their palates to dine regularly on small animals and freshwater fish, says Michael Richards of the University of British Columbia, Vancouver in Canada.

By studying the ratios of carbon and nitrogen isotopes from collagen in bones, Richards traced the main sources of dietary protein of 27 early Europeans and Neandertals; fish eaters, for example, have more nitrogen-15 in their bones than meat eaters. Richards found that the oldest known modern human in Europe—the 35,000-year-old jawbone from Peștera cu Oase cave in Romania—got much of his protein from fish. By 30,000 years ago, other modern humans got as much as 20% of their protein from fish. Meanwhile, the isotopes show that Neandertals in Europe stuck to meat from bigger animals, even when they lived at the same time in the same region as modern humans. This is the first direct evidence that Neandertals had a narrower diet, Richards reported in August in the *Proceedings of the National Academy of Sciences*.

The next big dietary shift came about 10,000 years ago, when humans began to domesticate plants and, later, animals. The move to agriculture introduced staples of the Western diet: cereal grains, sugars, and milk after weaning. For most of human evolution, our ancestors seldom ate these foods, says Cordain, who advocates avoiding refined grains and dairy products.

The agricultural revolution favored people lucky enough to have gene variants that helped them digest milk, alcohol, and starch. Those mutations therefore spread among farmers. But other populations remained more carnivorous, such as the Saami of frigid northern Norway, whose ancestors herded reindeer. Among Saami ancestors, genes to digest meat and fat efficiently were apparently favored. One gene variant, for example, makes living Saami less likely to get uric acid kidney stones—common in people who eat high-protein diets—than are people whose ancestors were vegetarian Hindus and lack this gene variant, says geneticist Mark Thomas of University College London (UCL).

But when ethnic groups abandon traditional lifestyles and rapidly adopt Western

*Evolution and Diseases of Modern Environments, at Charité University Medicine Berlin, Humboldt University, 12–14 October.

diets, they often suffer. Researchers have known for more than a decade that the Pima of the southwestern United States have “thrifty phenotypes”: sluggish metabolisms that store fat efficiently and boost survival on low-calorie diets. That’s probably because their ancestors in Mexico underwent frequent famine. When they eat the calorie-rich Western diet, the Pima develop high rates of obesity, diabetes, and high cholesterol, although their blood pressure stays relatively low.

Indeed, an epidemic of obesity is now spreading into ethnic groups from Siberia to Peru, as quickly as grocery stores open their doors. But although fast food is becoming the universal diet, not all people respond alike to this nutritional transition. For example, unlike the Pima, the Evenki reindeer herders and other indigenous peoples of Siberia have very high metabolisms, an adaptation to the cold that allows them to convert fat into energy efficiently. When the Soviet Union collapsed in the 1990s, many Siberians abandoned traditional lifestyles and diets. They too became obese and developed heart disease but in a different way from the Pima: The Evenki retained low levels of cholesterol and diabetes but developed high blood pressure, as University of Oregon, Eugene, anthropologist J. Josh Snodgrass reported recently in the *American Journal of Physical Anthropology*. In terms of disease risk, “the Pima are the mirror image of the Siberians,” says Leonard.

These disparate responses reflect a trend: In general, people who evolved in warm lowland environments where food may be scarce may have slow metabolisms for their body size, probably as adaptations to famine or heat stress; examples include the Pima and the Tsimane’ of lowland Bolivia. Groups that adapted to frigid or high-altitude climates, such as the Evenki or the Quechua of Peru, have high metabolisms, probably to convert fat into energy efficiently. Despite their differences, all of these groups risk disease when they switch to the Western diet. “They are telescoping into one generation trends that rolled out over a century or more in Western countries,” says pediatric nutritionist Jonathan Wells of the UCL Institute of Child Health.

From mother to child

Although we are what our ancestors ate, we are also what they didn’t eat. In India, for example, more than 66% of the population in some regions experienced famine during British colonialism a century ago. Women who survived tended to have low-birth-weight babies, whose bodies were small and

Paleolithic Diet

- High Protein (meat and fish)
- Low in Carbohydrates
- Tubers
- Nuts, Seeds, and Berries



Modern Diet

- High in Sugars
- Refined Carbohydrates
- Dairy
- High Fat



A tale of two diets. Our ancestors ate more protein and less dairy and refined carbohydrates than are included in the modern Western diet, which is also rich in sugar and fat.

efficient at storing fat, says Wells. It’s as though these babies took cues during fetal and early development about their mothers’ lifelong nutritional experience and adjusted their growth and body and organ size accordingly. Human stature often tracks the nutritional status of mothers, and it can take generations for descendants to recover. In India, average height in males dropped at a rate of almost 2 centimeters per century in the decades following colonialism, Wells reported online in October in the *American Journal of Human Biology*.



Fisheater. The owner of this jaw—the earliest known modern human in Europe—ate plenty of fish.

When these small babies gain weight in childhood, though, it stresses their smaller organs, such as the pancreas and heart, making them more susceptible to obesity, diabetes, and heart disease. This is the case in south India today, says Wells. There, many people have thrifty phenotypes with less muscle and more fat per body size. Yet they are shifting rapidly to a high-fat, high-sugar diet. As a result, Wells predicts, “India risks becoming the diabetes capital of the world.” Others agree: “I think there’s no question that people in south India are at higher risk,” says biological anthropologist Chris Kuzawa of Northwestern University, who says this is true of many other popula-

tions that have been poor in the past.

One way to prevent obesity and disease is to improve the diet of pregnant mothers and young children. But feeding pregnant women extra calories alone is not enough, warns Kuzawa, who says that a fetus takes in subtle cues about the quality of the mother’s nutrition, as well as her developmental history and that of her recent ancestors. He is part of a team exploring intergenerational effects in 3000 women in the Philippines.

Cordain proposed that everyone mimic a Paleolithic diet, adding protein and reducing refined carbohydrates and dairy, in a more extreme version of the low-carb Atkins-style diets. “It’s hard to get too fat on a diet without carbohydrates,” he says. He also cited a small study of 14 diabetic men in Sweden whose blood sugar improved on a diet free of carbohydrates and dairy products (*Science*, 13 July 2007, p. 175).

But other researchers argued that the problem is, simply, consuming too many calories. “What is clear is having low weight is positive,” says immunologist Andreas Pfeiffer of the Charité Berlin and the German Institute of Human Nutrition in Berlin. All agreed that controlled studies are needed to see whether all calories are alike or whether the same number of calories from protein, fat, or sugar have different effects.

Others noted that even if one paleodiet proves particularly healthy, it would be hard for people in different cultures to comply with it. “Food is identity,” says Ungar. “You can’t tell an Eastern European Jew to eat pork” or an Italian to skip pasta. The bottom line, says Leonard, is that although some diets are better than others, “there isn’t a perfect diet that is the same for everyone. The nature of our success is to find and make a meal in virtually any environment. But our different responses are structured by the basic biology we bring to the table.”

—ANN GIBBONS



LETTERS

edited by Jennifer Sills

Political Pressure's Effect on Repository Sites

R. C. EWING AND F. N. VON HIPPEL'S PROPOSAL FOR THE CONSTRUCTION of multiple repositories for spent nuclear fuel ("Nuclear waste management in the United States—starting over," Policy Forum, 10 July, p. 151) ignores history and current events. The 1982 Nuclear Waste Policy Act mandated that a search for a second repository be undertaken in the eastern half of the United States to complement the ongoing search in several western states, a search that led to Yucca Mountain, Nevada (1). The search in the eastern states, undertaken by the U.S. Department of Energy (DOE) in consultation with the states, was abandoned by the Reagan administration in response to intense

political pressure from a handful of states with sites deemed suitable for a repository. Similarly, the abandonment of Yucca Mountain did not result from the technical liabilities cited by Ewing and von Hippel, but rather from politicians reacting to an assortment of NIMBY ("not in my backyard")-related issues. In August 2008, the Directors of the DOE's National Laboratories, including current Secretary of Energy Steven Chu, jointly called for "licensing of the Yucca Mountain Repository as a long-term resource" [p. 6 in (1)]. These scientists were not unaware of the unresolved technical issues at Yucca Mountain—issues identified and addressed by their own staffs years ago—but they considered the technical assets of the site to more than compensate for the liabilities. Ewing and von Hippel underestimate the severity of NIMBY reaction to their proposal, even if funding (on the order of billions of dollars per site) could be found for the decade-long studies needed to evaluate each of their proposed regional sites.

So, what to do? To assure century-long security, transport the spent nuclear fuel to a military base, one encompassing a geologic environment warranting a pilot-plant study of subsurface disposal. The Nevada Test Site, the location during the Cold War of hundreds of underground nuclear weapons tests, remains a logical contender for spent fuel sequestration.

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Reference

1. M. Holt, Nuclear Waste Disposal: Alternatives to Yucca Mountain (Congressional Research Service, 6 February 2009).

Response

WINOGRAD BELIEVES THAT IT IS THE NIMBY ("not in my backyard") syndrome that has fueled political and public opposition to the siting of nuclear facilities, particularly the nuclear waste repository in Nevada. Our point, as explained in our Policy Forum, is that one must expect sustained political and public opposition such as NIMBY in the absence of a transparent process, strong public participation, careful attention and discussion of technical issues, a clear regulatory framework, and an appropriate management structure (not DOE). This past June, Sweden announced the location of its geologic repository for spent nuclear fuel, the result of a nearly two-decade open process with the exten-

sive involvement of the local communities.

Winograd cites the 2008 statement by the directors of national laboratories, including Steven Chu, then the director of Lawrence Berkeley National Laboratory, as an endorsement of Yucca Mountain. We note that it is the same Steven Chu, now the Secretary of Energy, who has removed Yucca Mountain from consideration as a nuclear waste repository, noting in testimony to the Senate Energy and Natural Resources Committee (5 March 2009) that "I think we can do a better job...." One interpretation of this change in position is that political considerations have again overwhelmed the process. As long as this remains the case in the United States, a prudent public may be wise in saying "not in my backyard."

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Not Much Novel Under the Sun

EARLIER THIS YEAR, WHEN THE *NEW ENGLAND Journal of Medicine* heralded the arrival of a new strain of pandemic flu, it did so in an article titled "Emergence of a novel swine-origin Influenza A (H1N1) virus in humans." The explosive and inappropriate use of the word



Yucca Mountain



Fast driving of electron spins in diamond

1489



DNA recognition code

1491

"novel" is itself spreading like a virus through the medical literature. To many students of English, referring to a virus as novel connotes the sense that its creation was associated with striking innovation and creativity. Unless the authors intended to make an argument about intelligent design or perhaps a heretofore undiscovered bioterror plot, it would seem more accurate to describe H1N1 as a new rather than a novel disease.

The questionable use of the word novel is not an isolated phenomenon. In 1970, 1 in every 2500 papers in the Medline database contained the word "novel" in its title. By 2007, use of the word had grown—1 in every 71 papers contained the word novel. And it's not just that new things are being discovered more frequently. The percentage of titles with the word "new" in them has remained relatively flat, hovering at about 2% of all Medline articles for the past 40 years. In 1970, we saw one "novel" discovery for every 50 "new" ones. By 2007, that ratio had changed so that a "novel" discovery came along for every 1.45 "new" discoveries.

What should we make of the steady rise in "novel" papers? We suggest that the trend represents two things. First, the growing ties between scientific and financial endeavors offer some explanation. United States legal codes list "novelty" as one of the conditions of patentability, thereby equating the word with commercially viable innovation. Of course, calling something novel does not make it patentable. The second likely explanation has been an increase in the degree to which authors of scientific papers resort to self-promotion and hyperbole in the effort to publish papers that will be noticed. Certainly, the growth in the sheer number of scientific articles that are published makes this tempting. If you want your papers to be read, there's nothing like a bit of self-aggrandizing puffery to try to stand out.

Whether the motives are commercial or professional,

however, scholarship is exactly what is at issue here. When scientists feel the need to resort to novel ways of saying "new," even at the price of misrepresenting their meaning, the effect is to diminish the standing of their work.

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Medical Report's Static Charge Sparks Shock

THE REPORT RELEASED BY THE ASSOCIATION OF American Medical Colleges (AAMC) and the Howard Hughes Medical Institute (HHMI) (1), which S. Long and R. Alpern extol in their Editorial "Science for future physicians" (5 June, p. 1241), received widespread press and recognition. However, it is based mostly on opinions that have weak support, and the radical changes proposed serve more to justify and perpetuate the existence of the AAMC and HHMI than to address actual problems in medical education.

The basis of the entire AAMC/HHMI report is the concern that the medical education process has been static.

There is no data to justify that this problem exists in medical education. In fact, teaching methods have evolved in medical schools throughout the country. Simulators are used far more frequently than in past years, as are interdisciplinary teaching and problem-based learning (2, 3). With the exception of the methods of standardized testing, which admittedly have remained unchanged, there is no reason to claim that education has been static.



Of the greatest concern is the self-admission in the report that the AAMC and HHMI have no way to assess what they perceive are necessary competencies in medical training. They are therefore suggesting drastic changes in education without knowing what they are trying to achieve. Quality improvement dictates that in order to improve a process, outcomes must be measurable and problems identified. The AAMC/HHMI document failed in this critical first step. This is the height of folly in quality improvement and in scientific method.

I credit any organization that is furthering the effort to enhance the training of new physicians in an ever-changing health care climate. However, it should be done rationally and not be based on the feeling that something should be changed merely for the sake of change.

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1. AAMC, "Scientific foundations for future physicians" (www.aamc.org/scientificfoundations).
2. G. Camp, *Med. Educ. Online* 1, 1 (1996).
3. P. Bradley, *Med. Educ.* 40, 254 (2006).

Response

KATZ RAISES TWO MAIN ISSUES CRITICAL OF our Editorial and the AAMC/HHMI report, "Scientific foundations for future physicians." First, the letter chastises the committee for making the claim that medical education has been static. The report does not make such sweeping statements, but focuses on the science component of medical education. The committee was well aware of the many educational innovations that have been instituted at medical schools to improve physician education, such as the use of simulators and problem-based learning. Unfortunately, few of these innovations have involved the content of science taught to medical students. Also of concern, and a focus of the committee, is the inability of undergraduate schools to innovate their science curriculum due to constraints placed on them by premedical course requirements and the MCAT examination.

The second point raised by Katz is the lack of outcomes. Unfortunately, there are no good outcomes to define the quality of medical education. Outcomes such as performance on standardized tests do not address whether we are training medical students optimally. We are not aware of any method that allows one to quantify the quality of physicians, the critical outcome measure. In the absence of a quan-

tifiable outcome measure, we asked 22 undergraduate and medical school teachers to define the optimal science education for physicians. It remains the view of our committee that a broad national effort is needed to increase the interdisciplinary nature of teaching and the mathematical and physical science interface with medicine.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

CORRECTIONS AND CLARIFICATIONS

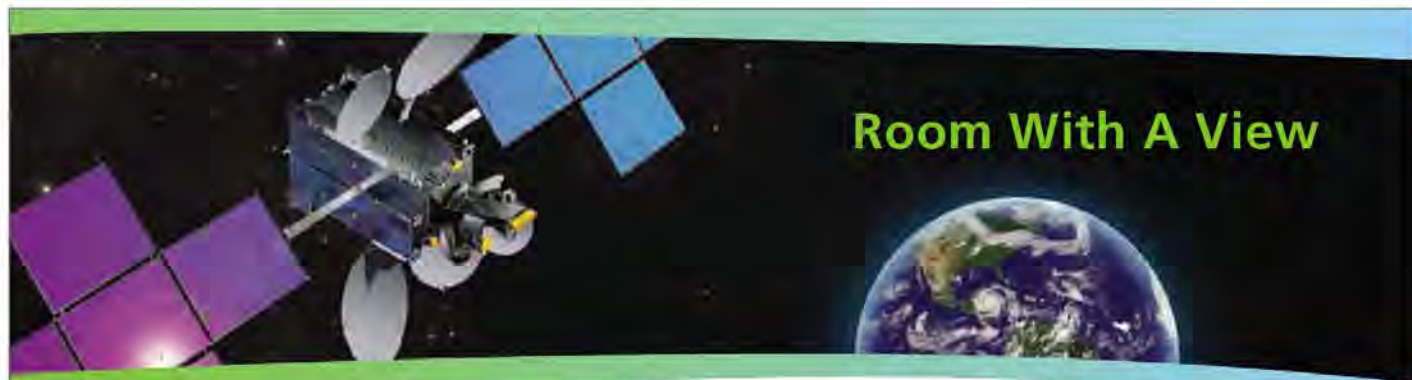
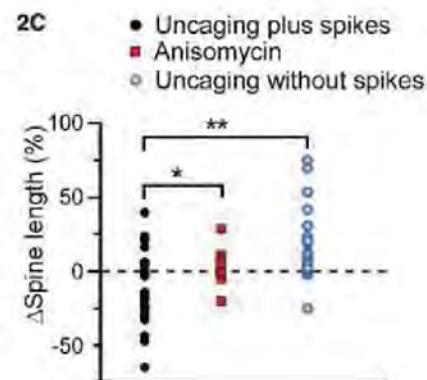
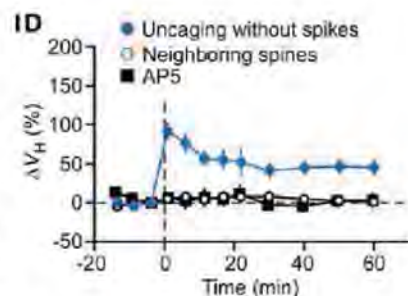
News Focus: "Seeking a shortcut to the high-energy frontier" by A. Cho (4 December, p. 1342). The story overstates by a factor of 1000 the estimated particle backgrounds that would be generated by radiation from the circulating muon beams. The correct figures are several hundred neutrons and tens of thousands of photons per square centimeter per bunch crossing.

Review: "Einstein's theory of gravity and the problem of missing mass" by P. G. Ferreira and G. D. Starkman (6 November, p. 812). The caption of Fig. 1 should have referred to the vertical line at $7 \times 10^{-8} \text{ cm s}^{-2}$, rather than to a horizontal line at $10^{-8} \text{ cm s}^{-2}$.

Perspectives: "Probing the cold Universe" by M. Rowan-Robinson (31 July, p. 546). In the first sentence of the first paragraph, the launch date of the Herschel and Planck satellites should have been 14 May 2009, not 14 April 2009. In the first sentence of the second paragraph, the position of the Lagrangian point was incorrectly described as between Earth and the Sun. That sentence should begin, "From its final destination at the Lagrangian point on the far side of Earth from the Sun...."

Reports: "Mammalian expression of infrared fluorescent proteins engineered from a bacterial phytochrome" by X. Shu *et al.* (8 May, p. 804). The affiliation for Antoine Royant was incomplete; he is with the Institut de Biologie Structurale UMR 5075 CNRS-CEA-UJF.

Reports: "Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines" by J. Tanaka *et al.* (21 March 2008, p. 1683). Figures 1D and 2C were incorrect; the corrected figures are shown here. In the legend for Fig. 2C, "Mann-Whitney *U* test" should be "Steel's multiple-comparison test." On p. 1685, right column, 10 lines from the bottom, "(6.7 ± 4.9%)" should be "(19.5 ± 5.8%)." On p. 1686, left column, 10 lines from the top, "(2.0 ± 1.9%)" should be "(4.8 ± 3.5%)."



Your mission is to understand and predict changes in Earth's environment. Our mission is to provide you with the access to space needed to fulfill your mission. That means getting your sensors and instruments into space quickly. Our pole-to-pole coverage helps you more accurately observe changes in the oceans, coasts, and atmosphere.

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FOR YOUNGER READERS

Enticing Tales of Science—Some Suggestions from 2009

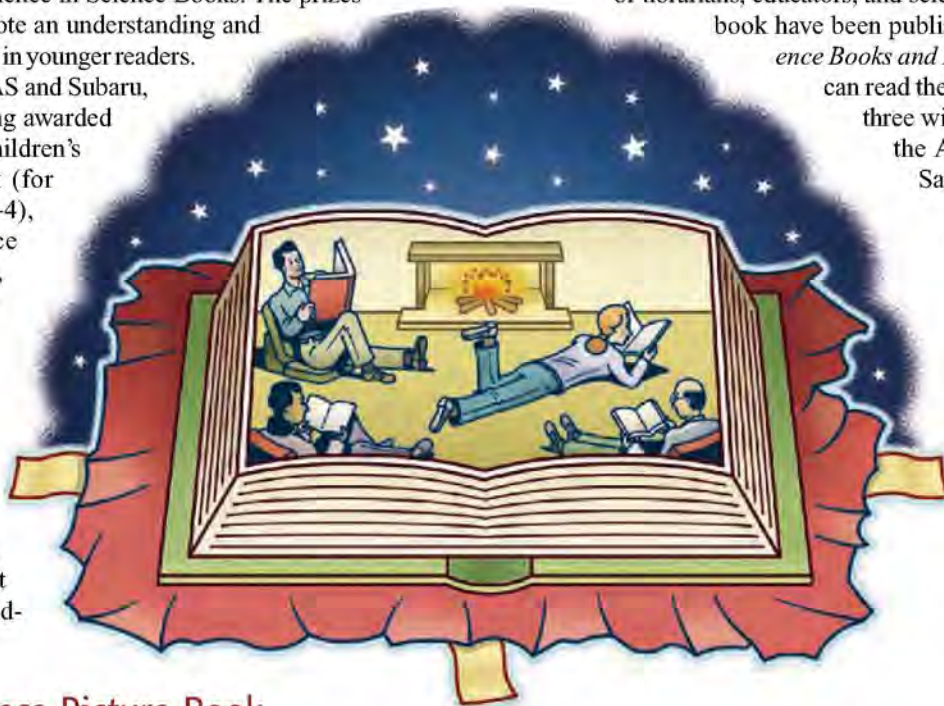
Engaging books are one way to encourage an interest in science in children and young adults. For some ideas for your holiday gift list, consider the finalists for the 2010 *Science Books and Films* Prizes for Excellence in Science Books. The prizes honor titles that promote an understanding and appreciation of science in younger readers. Sponsored by the AAAS and Subaru, this year they are being awarded in three categories: children's science picture book (for readers in grades K–4), middle grades science book (grades 5–8), young adult science book (high school). As is usually the case, the finalists for the young adult award are not books specifically intended for that age group—instead, they were written for the public at large. The titles consid-

ered for the 2010 prizes were published between September 2008 and August 2009.

Here, we offer short descriptions of the 13 finalists chosen by panels of librarians, educators, and scientists. Full reviews of each book have been published or will appear in *Science Books and Films*, and AAAS members can read these reviews on the Web. The three winners will be announced at the AAAS Annual Meeting in San Diego in February.

Among the grounds for selecting the prize winners are a clear and accurate presentation of scientific concepts. But we and the judges hope that the books will appeal to young readers so that they turn the pages for enjoyment as well as for what they may learn.

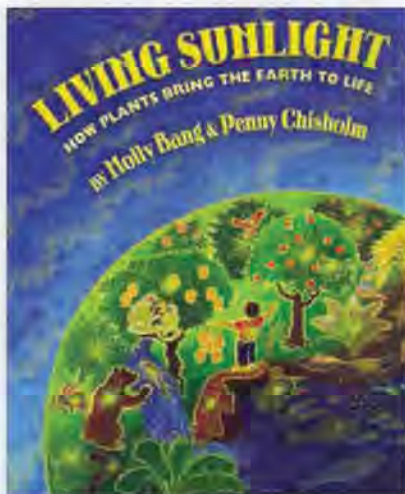
—Heather Malcomson,¹
Barbara Jasny, Sherman Suter



Children's Science Picture Book

Living Sunlight: How Plants Bring the Earth to Life. Molly Bang and Penny Chisholm. Blue Sky (Scholastic), New York, 2009. 36 pp. \$16.99, C\$18.99, £10.31. ISBN 9780545044226.

The Sun narrates a clear, lyrical account of photosynthesis and its fundamental roles in life on Earth. It sketches what happens at the molecular level as green plants capture solar energy in their chlorophyll, use it to break apart water, and build sugar from carbon dioxide. It explains that animals (people included) require the sugar and oxygen produced by plants, and that the carbon dioxide exhaled by animals when they combine these cycles back to the plants. Bang's vibrant illustrations—with leaves, animals, and landscapes outlined in bright yellow—light up the pages. Four pages of notes provide explanatory details (including the role of rubisco and chemical reactions for photosynthesis and respiration) and acknowledge some simplifications (such as leaving out phytoplankton, the focus of MIT ecologist Chisholm's own research). Effectively combining text and artwork, the authors offer a very enjoyable science lesson.



Moonshot: The Flight of Apollo 11. Brian Floca. Athenium (Simon and Schuster), New York, 2009. 44 pp. \$17.99, C\$19.99, £10.92. ISBN 9781416950462.

NASA's Apollo missions to the Moon captured the imagination of Americans and the entire world. Between 21 July 1969 and 14 December 1972, 12 astronauts set foot on the lunar surface. This book focuses on *Apollo 11*, the first mission to attempt to (and to) place humans on the surface. It is beautifully illustrated with large-size, full-color renderings of the participants, technologies, and wider physical and social environments within which the first Moon walk occurred.



Redwoods. Jason Chin. Roaring Brook, New York, 2009. 36 pp. \$16.95, C\$18.95, £10.21. ISBN 9781596434301.

While waiting for a subway train, a boy picks up an abandoned book and is drawn into a story about coastal redwoods. On the train, he reads that some redwoods now alive sprouted during the Roman Empire, and we see him sitting between a legionnaire and a citizen in a toga. He emerges from the subway to find himself in a redwood forest, where he learns about the growth of these tall trees, their resistance to fire, and their ability to make their own rain from the coastal fog. Pulling himself up a rope into the crown of a tree, he discovers the diverse fauna (birds, mammals, and amphibians) and flora (ferns, mosses, and

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REDWOODS

JASON CHIN



even other trees) that live in the forest canopy. From the height of skyscrapers, he rappels down into a city park. He notices the time and dashes off, leaving the book on a bench. A girl picks it up and starts off on her own adventure. Chin packs a great deal of information into his succinct text, and the blend of fantasy and realism in his watercolors will charm readers.

What Bluebirds Do. Pamela S. Kirby. Boyds Mills, Honesdale, PA, 2009. 48 pp. \$18.95, £11.50. ISBN 9781590786147.

North America is home to three species of bluebirds. Children who live near these beautiful birds are sure to enjoy the pages of Kirby's photo-

graphs showing the life of a family of eastern bluebirds that nested in her backyard. The author starts the story with the parents' courtship. They mate, build their nest in a tree, and hatch five eggs. The narrative is based on the author's actual observations, and in this case the female had to be taught by her partner how to care for the babies. The simple, appealing text presents many interesting facts—for example, as part of his efforts to find favor in the female's eyes, the male will hold a worm in its beak and sing at the same time. At the book's end, Kirby describes how bluebirds get through the fall and winter and lists other books and Web sites for additional information. Here, she stresses how providing nest boxes helps bluebird populations. I only wish she had included directions for making those birdhouses.

What Bluebirds Do



Middle Grades Science Book

Bodies from the Ice: Melting Glaciers and the Recovery of the Past.

James M. Deem. Houghton Mifflin, Boston, 2008. 64 pp. \$17. ISBN 9780618800452.

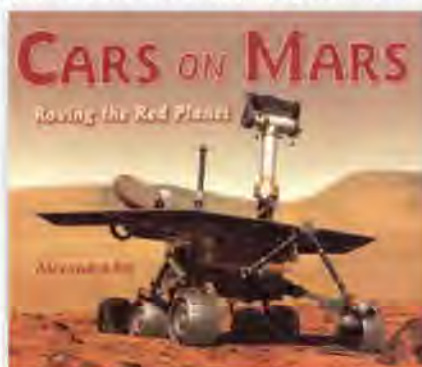


Preserved bodies frozen in ice present one aspect of human history that few people, other than a small number of specialists, ever glimpse. Deem has compiled a highly useful and readable book on that unusual aspect of prehistory. He pulls together detailed and well-illustrated accounts of the scientific investigation of such discoveries as the iceman Ötzi, the remains of frozen children in the Peruvian Andes, and the body of George Mallory on

Mount Everest. In addition, he provides a clear discussion of glaciology and how prehistoric events can be preserved. The short book accurately portrays the process of science from discovery to investigation.

Cars on Mars: Roving the Red Planet. Alexandra Siy. Charlesbridge, Watertown, MA, 2009. 64 pp. \$18.95, £12.99. ISBN 9781570914621.

At the end of the book, Siy says that she fell in love with Mars in 2003, when she viewed the shimmering planet while on a camping trip. That love is apparent in her history of NASA's Mars Exploration Rovers. She starts with Cornell University astronomer Steve Squyres and his dream of exploring Mars to learn about its past history and to look for signs that life may have existed there. The author describes the landing of the two golf-cart-sized rovers (Spirit and Opportunity) in January 2004 and the trials and triumphs of their subsequent travels across the planet's surface. Through the story, readers will learn



about the design of the rovers, the scientific questions that their missions have addressed, and the challenges Earth-bound researchers faced to keep them sending back information. The very readable text and excellent photographs (made available to the public by NASA, JPL-Caltech, Cornell, and the U.S. Geological Survey) depict a scientific and technical triumph.

The Frog Scientist. Pamela S. Turner, photographs by Andy Comins. Houghton Mifflin, Boston, 2009. 64 pp. \$18, £10.92. ISBN 9780618717163. Scientists in the Field.

As a child in South Carolina, Tyrone Hayes liked to collect frogs, snakes, and turtles. As a professor at the University of California, Berkeley, his research focuses on the effects of steroid hormones on amphibian development. (These may contribute, along with factors such as habitat loss and a devastating fungal disease, to the drastic global decline in amphibians.) Turner joins Hayes and his students in the field (a Wyoming pond) and laboratory while they test the hypothesis that the commonly used pesticide atrazine feminizes male leopard frogs. She describes how they design, carry out, analyze, and interpret their experiment. She also tells how Hayes struggled as an undergraduate at Harvard until he found his calling in a research lab. The book introduces colorful frogs and toads from around the world as well as some of the efforts to save them from extinction. The lucid text and numerous photos of Hayes, his young assistants, and amphibians combine to convey both the fun and importance of doing science.



Lucy Long Ago: Uncovering the Mystery of Where We Came From.

Catherine Thimmesh. Houghton Mifflin, Boston, 2009. 64 pp. \$18, £10.92. ISBN 9780547051994.

The award-winning author's latest engaging book tells the story of the discovery of a fossil hominid and what that finding can tell us about our ances-

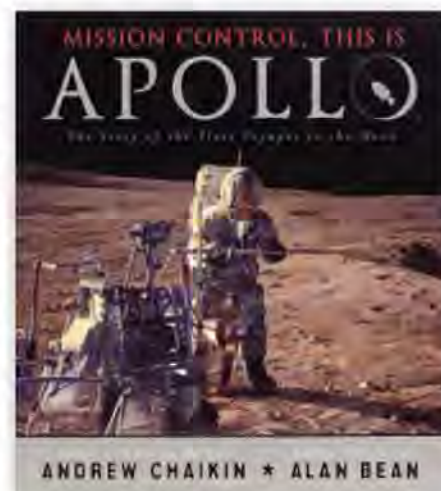


tors and ourselves. Each chapter poses a question—"Child or grownup?" "Boy or girl?" "Ancient or modern?" "Known species or new?"—and proceeds to describe how paleontologists answered these questions through their work. Through these, Thimmesch demonstrates perfectly how the scientific method can be used to answer such questions. Photographs from the field and laboratory, graphs, charts, and computer-generated pictures of Lucy in her ancient habitat add to the book's appeal.

Mission Control, This Is Apollo: The Story of the First Voyages to the Moon. Andrew Chaikin and Alan Bean. Viking, New York, 2009. 128 pp. Paper, \$23.99, C\$30, £14.56. ISBN 9780670011568.

Forty years after the first landing on the Moon, the authors trace the history of the 20th century's greatest adventure. Although intended for children

ages 8 to 12, their accurate account will delight and inform readers of all ages, from those who remember the excitement of Apollo to those who have only heard about it. The well-illustrated book includes many paintings by Bean, an Apollo 12 astronaut and now a professional painter. Chaikin, Kohl, and Bean describe the hazards, drama, danger, and rewards of exploration. Their discussion of the risks faced by the astronauts is particularly relevant as NASA prepares to return to the Moon in an era when the public seems much less willing to accept the loss of lives in space.



Young Adult Science Book

Confessions of an Alien Hunter: A Scientist's Search for Extraterrestrial Intelligence. Seth Shostak. National Geographic, Washington, DC, 2008. 319 pp. \$27, C\$32, £14.99. ISBN 9781426203923.

Shostak recounts the search for signs that intelligent life exists on a planet other than Earth—from the conceptions of the Greek philosophers to radio astronomers and the ongoing Search for Extraterrestrial Intelligence (SETI).

Researchers have been searching, for decades, for radio transmissions that would represent evidence of intelligent life on another planet. As a senior astronomer at the SETI Institute in California, the author is certainly an enthusiast. He is, however, careful to inject an appropriate amount of balance: He describes false leads that have set scientists' hearts pounding (no matter how briefly) and reasons why some scientists believe that intelligence anywhere else in the universe would be an extremely rare event. He debunks the idea that aliens have visited us already even while considering what our reaction will be when we find them. There are provocative discussions of the likelihood that we will first

make contact with alien machines rather than biological creatures. In addition to these enticing speculations, readers will also find informative coverage of the basics of astronomy and the components of ancient life on Earth. Shostak tells this fact- (and idea-)filled story with a wry sense of humor.

The Invisible Kingdom: From the Tips of Our Fingers to the Tops of Our Trash, Inside the Curious World of Microbes. Idan Ben-Barak, Basic, New York, 2009. 214 pp. \$24, C\$30.50. ISBN 9780465018871. **Small Wonders: How Microbes Rule Our World.** Scribe, Carlton North, Australia, 2008. Paper, 240 pp. \$A26.95. ISBN 9781921372179.

Biofilms, antibiotic resistance, horizontal gene transfer, and the value of hand washing are among the vast range of topics Ben-Barak discusses in this



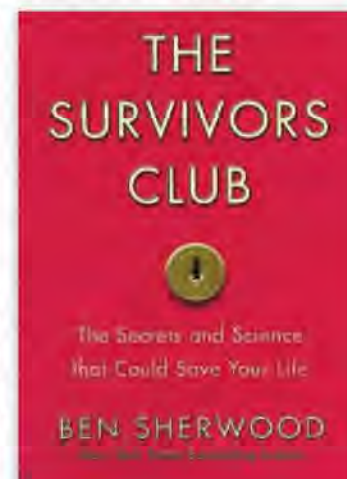
introduction to microbes. His old and loose definition of the term encompasses bacteria, archaea, fungi, and protists. He also considers forms from the untidy border of life itself: viruses, transposons, prions, and Tasmanian devil tumor cancer. The fact-packed chapters reveal how microbes develop, behave, and evolve and how life on Earth would cease without them. "Bonus tracks" offer additional fascinating stories, such as that of the "corrupted blood" epidemic in the virtual World of Warcraft. The author often mentions how scientists

have developed our understanding of microbes, and he frequently raises unanswered questions and conflicting interpretations. Readers will find his chatty and humorous text offers a fun way to acquire a vast store of intriguing details about the small wonders that inhabit our world and our bodies.

The Survivors Club: The Secrets and Science That Could Save Your Life.

Ben Sherwood. Grand Central, New York, 2009. 399 pp. \$25.95, C\$28.99. ISBN 9780446580243. Michael Joseph (Penguin), London. £15.99. ISBN 9780718153106.

Sherwood combines true stories and scientific research to explain why some people, but not others, survive disasters, diseases, and accidents. The story-driven narrative makes his book one that young adults will read cover to cover. The author interviews numerous people who have survived a variety of events (moun-



tain lion attack, car versus bike accident, cancer, prisoner of war, and holocaust) to determine why they did so. He alternates these accounts with scientific perspectives and interviews of researchers who have extensive experience in the field of survivor psychology. He leaves readers with some valuable techniques for improving their odds of making it through dangerous situations (anticipate, prepare, keep cool, be proactive).

Why Sh*t Happens: The Science of a Really Bad Day.

Peter J. Bentley. Rodale, New York, 2009. 320 pp. \$16.95.

ISBN 9781594869563. **The Undercover Scientist: Investigating the Mishaps of Everyday Life.** Paper, Arrow (Random House), London, 2009. £8.99. ISBN 9780099522423.

Throughout this definitely quirky book, computer scientist Bentley is talking directly to you. And you are having a truly harrowing day: You get your finger stuck in a bottle, start a fire accidentally in your microwave, superglue



happens, you do survive the day. You probably will get many chuckles (and some insight) from reading the book.

10.1126/science.1184317

For Younger Readers

Two from Japan

Carl Kay

To broaden the geographic scope of our coverage, we sought suggestions from Hiroe Masumoto, head of the Children's Science Books Committee of the Japan Association for the Study of Child Literature—a nonprofit group of librarians, teachers, authors, and others who study and review children's books (<http://homepage3.nifty.com/kodomonohonken>). The first of her two recommendations is a picture book; the second is appropriate for readers in the middle grades.

Hebi no Himitsu [Secrets of Snakes]. Ryu Uchiyama. Popular Publishing, Tokyo, 2008. 36 pp. ¥2310. ISBN 9784092131910. Fushigi Ippai Shashin Ehon [Truly Amazing Photo Books for Kids], 14.

Young readers will be enthralled by the author's stunning close-up photographs and simple text. These convey the beauty, power, and abilities of snakes as well as the "secrets" of their survival. For example, snakes smell and feel with their tongues, and they hear with ears hidden from our view. Many have flexible jaws that allow them to swallow volumes several times the size of their head. Some species even have a bone inside their body for breaking the eggs that they devour whole with these jaws. The more than 50 photographs show snakes shedding their skin, hatching, and capturing and swallowing prey. Some illustrate details of snake anatomy (such as where their bodies eliminate urine, which quickly turns to a white chalk). Lest children find these fascinating animals too attractive, Uchiyama includes a short warning on the danger of poisonous snakes.

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Noasobi Wo Tanoshimu Satoyama Hyakunen Zukan [Enjoying Nature Close at Hand: An Illustrated Guide to Our Enduring Satoyama].

Tatsuhide Matsuoka. Shogakukan, Tokyo, 2009. 36 pp. ¥1200.

ISBN 9784591107485.

The landscape system called satoyama, literally "village and mountains," mixes secondary woodlands, grasslands, wetlands, fields, orchards, rice paddies, farmsteads, and villages in a complex mosaic. Here, humans interact intimately with the nature around them without either destroying it or walling it off into untouchable preserves.

(Through the Satoyama Initiative, the Japanese Ministry of the Environment is working to preserve such landscapes, because they offer a balance between conserving biodiversity and the sustainable use of resources.) The author's hundreds of beautiful drawings capture the feeling of today's elders in Japan who grew up gathering mushrooms and berries, fishing, drying flowers, and chasing fireflies even in areas not far from urban centers. The text describes the daily activities of birds, plants, insects, and humans in these settings. There's even a recipe for a mixture of banana, whiskey, and sugar to brush on a tree trunk to attract night-flying insects.

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LAND USE

Sustainable Floodplains Through Large-Scale Reconnection to Rivers

Jeffrey J. Opperman,^{1,2*} Gerald E. Galloway,³ Joseph Fargione,¹ Jeffrey F. Mount,² Brian D. Richter,¹ Silvia Secchi⁴

If riverside levees are strategically removed or repositioned, the result can be reduced flood risk and increased goods and services.

Flooding is the most damaging natural disaster worldwide, and the flood-vulnerable population is expected to grow in coming decades (1). Flood risks will likely increase because of both climate change (1) and shifting land uses, such as filling of wetlands and expansion of impervious surfaces, that lead to more rapid precipitation runoff into rivers. In the United States, annual river flood losses continue to rise (2), punctuated by major events in the Midwest (1993, \$30 billion in total costs; 2008, \$15 billion) and California's Central Valley (1995 and 1997; \$4 billion each event) (3). Meanwhile, pressure to develop new housing in flood-prone areas near rivers (floodplains) continues (4), even as levee-system maintenance is chronically underfunded (5).

Flood-control infrastructure (e.g., levees) prevents high flows from entering floodplains, thus diminishing both natural flood-storage capacity and the processes that sustain healthy riverside forests and wetlands. As a result, floodplains are among the planet's most threatened ecosystems, even though functioning floodplains—those connected to rivers—are among the most valuable ecosystems for supporting biodiversity and providing goods and services to society (6, 7). We propose that a large-scale shift in land use and policy is urgently needed to achieve economically and environmentally sustainable floodplain management. The area of floodplains allowed to perform the natural function of storing and conveying floodwaters must be expanded by strategically removing levees or setting them back from the river.

Floodplain reconnection will accomplish three primary objectives: flood-risk reduction, an increase in floodplain goods and services, and resiliency to potential climate-change impacts. Efforts should focus on strategic reconnection of large areas of floodplain



The Yolo Bypass while flooded. [Photo by William Harrell, California Department of Water Resources]

currently used for agriculture, as large-scale reconnection of densely populated floodplains would be considerably more expensive. The changes we propose will confront considerable socioeconomic and political challenges, but we believe these can be overcome by promoting floodplain land uses that are consistent with private ownership and a vibrant agricultural economy. Although our specific recommendations are for the United States, this vision is applicable worldwide. Similar calls for change have been made in several countries [e.g., (8)].

Reduced Risk, Enhanced Benefit

Large-scale floodplain reconnection will reduce flood risk in two ways. First, land use within reconnected floodplains will move toward activities compatible with periodic inundation. Flood-tolerant land uses (described below) will be much less vulnerable to flood damages and therefore less likely to require disaster relief payments. Second, reconnection increases the area available to store and convey floodwaters and can reduce flood risk for nearby areas. In most of the United States, this benefit occurs haphazardly

through levee failure. For example, during 2008 floods in the U.S. Midwest, a town was spared because a nearby levee protecting croplands failed, allowing floodwaters to inundate fields and alleviating pressure on the town's levees (9). But strategic reconnection of floodplains, designed and implemented to maximize public-safety benefits, holds great promise for reducing local and regional flood risk (8). For example, a study of the Illinois River found that reconnection of 8000 hectares (ha) of floodplain would improve protection for 26,000 ha of farmland by halving the probability of inundation from major floods (10).

Large-scale reconnection of floodplains may also increase flexibility and resilience of water-management infrastructure. Globally, thousands of large, multipurpose dams provide (or are being built to provide) flood control and water supply and/or hydropower. The need for partially empty reservoirs (to store floodwaters) must be balanced with the benefits from full reservoirs (water supply, hydropower, recreation, and environmental flows to maintain healthy ecosystems). Climate-change models suggest that many regions of the world will experience increased frequency

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of both floods and droughts, exacerbating the challenge of balancing these multiple objectives (1). Large-scale floodplain reconnection provides floodwater storage and conveyance, reducing the need for upstream reservoirs to remain partially empty and thus increasing the benefits they could provide when full. Increased resiliency of water management systems through floodplain reconnection is a promising example of ecosystem-based adaptation to climate change.

A key to the economic and political viability of this approach is that reconnected floodplain areas can remain largely under private ownership, generating revenue such as through productive agriculture. Agricultural practices consistent with periodic inundation include pasture, timber, and cultivation of flood-tolerant crops (e.g., biomass fuel sources such as switchgrass and willow) (11). Where there is strong seasonality in floods, such as the western United States, annual crops can be grown during the dry season. Even where floods are coincident with the growing season, a broader variety of crops could be cultivated on reconnected lands that are inundated less frequently (e.g., less than once per decade).

Connected floodplains under natural vegetation support high levels of biological productivity and diversity (7) and provide numerous ecosystem services, second only to estuaries among ecosystem types in terms of value per hectare (6). For example, perennial plants, in either restored habitats or biomass crops, increase carbon sequestration (12) and improve water quality by reducing soil erosion and increasing nutrient retention (11).

Funding Management and Reconnection

The vision outlined here is based on the premise that new floodplain land-use patterns, allowing periodic conveyance and storage of floodwaters, will produce substantial societal benefits, including reduced flood risk or increased benefits from multipurpose reservoirs. Achieving these benefits will incur large upfront costs for levee setbacks, flow easements, land acquisition, and restoration, along with periodic compensation for flood damages. State or federal governments will need to support institutional or financial mechanisms that link floodplain landowners with beneficiaries of reduced flood risk, reservoir reallocation, and ecosystem services.

For example, beneficiaries (or, by proxy, government) could compensate landowners for increased flood risk or lost economic productivity on reconnected lands, similar to a proposal from the Sacramento Area

Flood Control Agency to compensate farmers whose land floods during large storms, serving as "relief valves" and easing pressure on developed areas (13). Ecosystem services could potentially generate revenue through emerging markets for carbon and nutrient sequestration. Other services—such as providing wildlife habitat, open space, or groundwater recharge—can be supported by public sources of funding (e.g., Wetland Reserve Program), or, for example, through hunting leases and "banks" for wetlands, endangered species habitat, or groundwater (14, 15).

As an indication of the potential demand for floodplain reconnection, the U.S. Department of Agriculture (USDA), which received \$145 million under the American Recovery and Reinvestment Act to acquire floodplain easements, received applications for >10 times the area of land (192,000 ha) than they were able to enroll (14,400 ha) (16). Current legislative and policy-making processes for water [e.g., forthcoming revisions to the federal Principles and Guidelines for water development (17)], as well as energy, agriculture, and climate change provide opportunities to promote floodplain reconnection as a flood-risk reduction tool.

Demonstrating Success: The Yolo Bypass

Although to date rarely implemented, this vision of large-scale floodplain reconnection is not unprecedented. California's Yolo Bypass conveys 80% of Sacramento River floodwaters during large events, routing water away from the city of Sacramento (see figure, page 1487) (18). The bypass was created in the 1930s by reconnecting a 24,000-ha floodplain when it became apparent that a "levees only" approach would not sufficiently reduce flood damages (19). By conveying large volumes of floodwaters, the bypass increases the flexibility of California's water management infrastructure. During a March 1986 flood, the bypass conveyed ~12.5 billion cubic meters (bcm) of water, more than three times the total flood-control storage volume in all Sacramento basin reservoirs (3.5 bcm). This occurred during a period when the flood-control system was operating near maximum capacity (20). Without the bypass floodplain, California would need to build massive additional flood-control infrastructure or allocate more of its already strained water-supply storage capacity to flood control.

Two-thirds of the bypass is privately owned, productive agriculture. During inundation, the bypass provides habitat for birds and native fish (18). The bypass provides

additional ecosystem services, such as open space for a rapidly growing region, recreation (including revenue-producing duck-hunting clubs), and groundwater recharge (of great value as a water bank during droughts) (14).

Conclusion

Unsustainable floodplain land use is common throughout the industrialized world, and developing countries are following the same trajectory (7). The vision outlined here is not a call to empty the floodplain of human activity. Rather it is an approach that would reduce unsustainable uses while maximizing floodplain benefits for both society and private landowners.

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PHYSICS

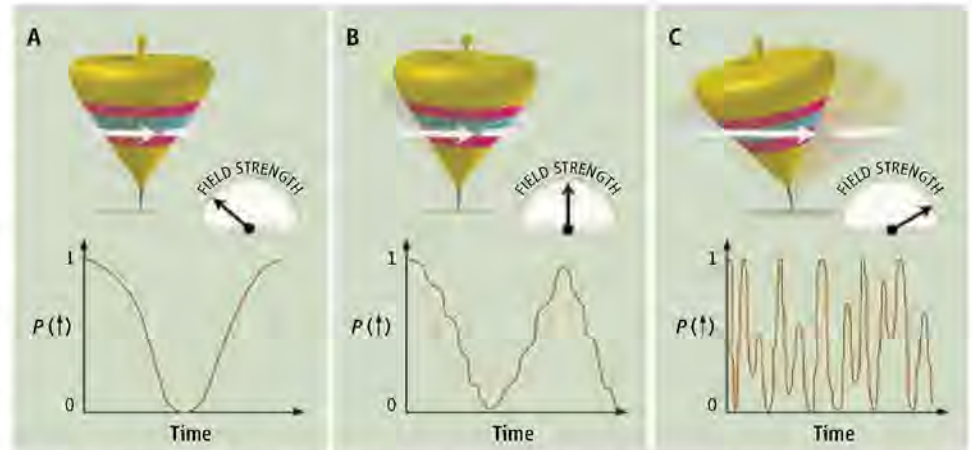
A Strongly Driven Spin

Brian D. Gerardot and Patrik Öhberg

Quantum information processing has the potential to open new horizons in fundamental science, communication, and computing. However, a number of experimental challenges must be overcome to realize this potential. Of primary importance is controlled manipulation of a qubit—the elementary building block of quantum information. A qubit is simply any two-level quantum mechanical system. In many solid-state materials, qubits—whether based on spin states, electrostatic charges, or some other property—suffer unwanted coupling with the surrounding environment. Such interactions lead to decoherence and degrade the information. Implementing realistic quantum information processing requires manipulating the two-level system at a rate much faster than decoherence occurs. On page 1520 of this issue, Fuchs *et al.* (1) take an important step in this direction by strongly driving a single electron spin in diamond. In the process, they surpass the limits of a widely used approximation—the rotating wave approximation (RWA)—and open new avenues for exploring quantum dynamics.

In a static, external magnetic field B_{ext} , an electron's spin-up and spin-down states are energetically split—an effect called Zeeman splitting. This two-level spin state can be manipulated with an applied electromagnetic field, typically in the microwave region, that has nearly the same energy as the Zeeman splitting. The microwaves create an oscillating magnetic field B_{ac} , which, if orientated perpendicular to B_{ext} , can coherently flip the electron spin state. However, the rate at which the spin state flips is not solely determined by the driving amplitude, as shown by Fuchs *et al.*

Although quantum phenomena are often highly counterintuitive, Feynman *et al.* (2) showed that the dynamics of a driven qubit are equivalent to torque on a spinning top. This torque vector can be decomposed so that, in a rotating frame of reference, there will be a “counterrotating” term which spins at twice the driving frequency, but in the opposite direction. In the driven qubit analogy, the “corotating” terms arise from the microwave driving field (H_1) and the “counterrotating” terms arise from the electron



Dynamics of spinning tops and resonantly driven electrons. (A and B) Most studies involving two-level system manipulation are performed with driving field strengths H_1 that are much weaker relative to the energy difference between the levels. In this regime, the two-level dynamics correspond to that of a fictitious spinning top, whose rotation frequency is proportional to H_1 . For an electron spin in an external magnetic field, the spin population [expressed as the population of spin up, $P(\uparrow)$], either varies smoothly (A) or exhibits small variations corresponding to the top slightly wobbling (B). (C) Fuchs *et al.* explored the regime of an electron spin where H_1 is comparable to the energy difference of the spin states. The complex dynamics correspond to a highly unstable spinning top. However, the inherently faster spin dynamics potentially allow much faster control. In their experiment, the spin could be controllably flipped in less than 1 ns.

Larmor precession frequency (H_0) caused by the Zeeman splitting.

This approach might seem unnecessarily complicated, but in the case where the amplitude of the driving field is weak compared to the Larmor precession frequency ($H_1 \ll H_0$), the counterrotating torque component changes direction so rapidly that it averages to zero. Hence, in this regime the counter-rotating term can be neglected—this is the rotating wave approximation. In this scenario, the spinning top is stable, and the change in the population from spin up to spin down has a smooth sinusoidal shape (see the figure, panel A), or, at modestly high fields, causes a small degree of wobble and only a small perturbation (see the figure, panel B). These stable limits have been exploited in optical and spin resonance spectroscopy.

However, processing quantum information is facilitated by manipulating the qubit as fast as possible, which corresponds to the strong driving limit. Unfortunately, as H_1 becomes comparable to H_0 , the counter-rotating term cannot be ignored and the RWA fails. The counterrotating term now imparts a highly nonlinear torque vector that forces the spinning top to wobble—equivalent to rapid swings in the spin state population (see the figure, panel C).

Extremely fast manipulation of an electron spin at a defect site in diamond may enable advances in quantum information science.

Fuchs *et al.* investigate a nearly ideal two-level system: an electron spin at a nitrogen-vacancy (NV) complex in diamond. The NV complex is a nitrogen impurity next to a void in the diamond crystal lattice. The electron spin on an NV center is remarkably coherent and well isolated from its environment even at room temperature (3). Moreover, optical transitions can be used to initialize (or preferentially populate) and subsequently identify the spin state.

The sample used by Fuchs *et al.* contained a dilute concentration of NV centers which allowed optical isolation of a single center. High-power microwave pulses (up to ~1 W per pulse) were delivered via an electromagnetic waveguide deposited on the sample surface. Because of diamond's amazing spin coherence properties, the experiment could be performed at room temperature. A small, static external magnetic field ($B_{\text{ext}} = 850$ G) was used to Zeeman split the spin states, causing the electron spin to precess ($H_0 = 490$ MHz).

The first step in the experiment was to initialize the spin state in one direction with a laser pulse. After a short pause, a 10-ns pulse of microwave radiation was launched down the waveguide to create B_{ac} . After another pause, the spin state was optically measured by monitoring the luminescence from a second laser pulse. This procedure

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was repeated $\sim 10^5$ times to achieve a suitable signal-to-noise ratio.

Smooth sinusoidal population oscillations were observed when $H_1 \ll H_0$, as observed previously with NV centers (4) and quantum dots (5). However, when Fuchs *et al.* increased H_1 , the sinusoidal curve displayed increasingly anharmonic components. Finally, at H_1 values nearly as large as H_0 the response became altogether nonlinear. In fact, at some time points, the spin state flipped much faster (< 0.5 ns) than would be possible in the valid RWA regime, whereas at other time points, the spin rotation appeared to stall. These outcomes are a direct result of the competition between H_1 and H_0 .

This approach could be used as a tool for probing fundamental physics, for example, in quantum control theory (6). With further optimization, this experiment provides a way forward for ultrafast control of a qubit for quantum information processing. A crucial metric in this endeavor is the number of times a qubit can be manipulated before its information dissipates. Ultrafast qubit manipulation is more straightforward for transitions with a large energy splitting (and hence large H_0) using laser pulses in the valid RWA regime—as shown for optically manipulated spins in quantum dots (7). However, the extraordinary spin coherence in diamond justifies the effort by Fuchs *et*

al., who demonstrated that more than 1 million qubit operations are possible at room temperature before decoherence occurs.

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ASTRONOMY

Two Missions, One Microquasar

Giovanni F. Bignami

It has been on the suspect list for more than 30 years, but now we know for sure, smoking gun and all, that x-ray source number 3 in the constellation of Cygnus (Cyg X-3) emits gamma rays. After many near (and wide-of-the-mark) misses, we now have incontrovertible evidence coming from two independent gamma-ray telescopes, AGILE (1) and Fermi (2), the latter reported on page 1512 of this issue.

The data were accumulated over the past 2 years, during which the object—an x-ray binary with a short (4.8 hour) orbital period, no pulsations, and strong, erratic radio emission—underwent several of its x-ray and radio ups and downs. It now appears that its 100 MeV gamma-ray emission is somehow linked to these modulations. It may turn out that gamma rays and the particles producing them hold the key to this peculiar x-ray binary, which, in modern parlance, is referred to as a “microquasar.” Like quasars, Cyg X-3 could contain a small accreting black hole, and it has relativistic jets.

A strange source then, Cyg X-3. Given the spartan facilities aboard an Aerobee rocket, back in the presatellite stone age of x-ray astronomy, the initial report of its discovery in 1966 (3) is to be read with reverence. Because Cygnus is located in a difficult-to-probe region of the Galaxy, it took all of 30

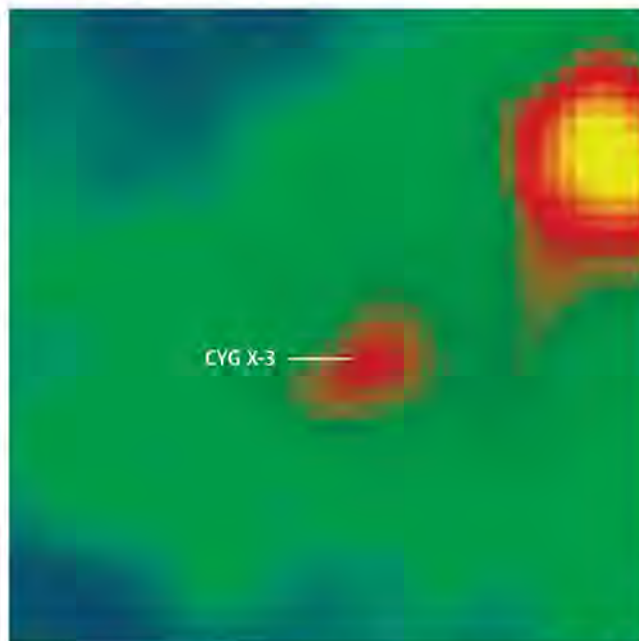
years for understanding the nature of the non-collapsed object in the system (4).

Meanwhile, observations (correct and incorrect) accumulated at all wavelengths, from radio to PeV gamma rays, and even (unconfirmed) cosmic rays. In the early 1970s, NASA's small astronomy satellite-2 (SAS-2) staged a claim for a positive Cyg X-3 detection. That claim, however, was not confirmed either by the European Space Agency's (ESA) Cosmic ray satellite (Cos-B) or by NASA's Compton Gamma-Ray Observatory (CGRO). For decades, conventional wisdom followed a simple model of the source as a young neutron star in a binary system (5), where the gamma rays were a result of particles accelerated at a shock discontinuity.

Because none of the observational high-energy claims stood the test of time, work concentrated on the radio-to-x-ray phenomenology of Cyg X-3. The object itself must take the blame for part of the difficulties for the high-energy detections of Cyg X-3. This binary, or microquasar, spends most of its time in states characterized by strong “hard” x-ray emission (tens of keV) and by intermediate values of radio

The detection of high-energy gamma rays is providing a better picture of the structure and dynamics of microquasars.

emission. Photons are somehow “killed” above 1 MeV, and no gamma rays can thus be detected. It is only at special moments, when radio emission is quenched and hard x-rays disappear into soft ones, when Cyg X-3 can unleash itself and seriously accelerate particles. It is the interaction of such particles (electrons or protons) that produce gamma rays. In short, observing a microquasar for a year is like observing a quasar for a million years.



Coming into view. The detection of gamma rays by the space-based satellites Fermi LAT (2) and AGILE (1) is providing a clearer picture of the microquasar Cygnus X-3.

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All of the above, however, remained a somewhat fuzzy and unsatisfactory model until a new generation of gamma-ray missions came along to prove it, starting a few years ago. Back in 2003, at the low-energy end, ESA's *Integral* satellite reported a solid observation up to 100 keV (6). But the breakthrough came with the 100-MeV range, which took on new vigour with the launch of ASI's (the Italian Space Agency) *AGILE* in 2007 and of NASA's *Fermi* in 2008.

The *Cyg X-3* paper by *AGILE* (1) refers to observations of the source over about 2 years (2007 to 2009). On four occasions during that period, *AGILE* saw gamma rays coming from the source at precisely those transitional states referred to above, that is, when the radio and hard-ray emissions appear to be suppressed. Of equal interest is that, on those special occasions, the gamma-ray-emitting state was followed by those strong radio jets for which *Cyg X-3* is famous. It stands to reason that relativistic (radio) jets can only be formed when

relativistic particles are being accelerated, and thus when gamma rays are being produced. A palpable hit, at last, for gamma-ray astronomy.

A similar long-term correlation between radio and gamma ray emission has been found by the *Fermi* Large Area Telescope (LAT) (2), an instrument that allows for a much richer photon harvest than *AGILE*. The LAT detects *Cyg X-3* at the right position with a significance of 29 standard deviations. Moreover, it detects the source 4.8-hour orbital periodicity, and does so during "active" source times when the accelerator is "on" and gamma rays are being copiously produced. They also find excellent correlation between the radio and gamma-ray emissions.

As for the source physics, there are a couple of clues. One is the gamma-ray spectrum of *Cyg X-3*, which appears to be steep. Electrons would be favored as the parents of such a variable, steep spectrum, especially if they were located away from the accretion disc and thus see the stellar radiation field as anisotro-

pic. The second clue, reported by *AGILE* and confirmed by LAT, is that the active periods seem to lead the radio ones by a few days.

Of course, *Cyg X-3* gamma rays could instead be produced by protons. If the dense environment of a microquasar can seriously accelerate protons, as some have claimed, and if we had available adequate neutrino detectors, we could be facing a candidate for post-electromagnetic astronomy. Now, that would be real fun.

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PLANT SCIENCE

DNA Binding Made Easy

Daniel F. Voytas¹ and J. Keith Joung^{2,3,4}

All cells encode specific DNA binding proteins that ensure that genetic material is appropriately expressed, replicated, and transmitted from one generation to the next. Mother Nature solved the DNA recognition problem by inventing a handful of protein motifs, including the zinc finger, the helix-turn-helix, and the leucine zipper. As is the case with all good solutions to a problem, these motifs are used over and over again in biological systems; for example, DNA binding proteins containing the helix-turn-helix motif are found in both prokaryotes and eukaryotes, and zinc finger-containing proteins are the most abundant protein class encoded by the human genome. It is surprising, therefore, to learn from studies by Boch *et al.* (1) on page 1509 and Moscou and Bogdanove (2) on page 1501 of this issue, about a new DNA binding motif that has heretofore escaped description.

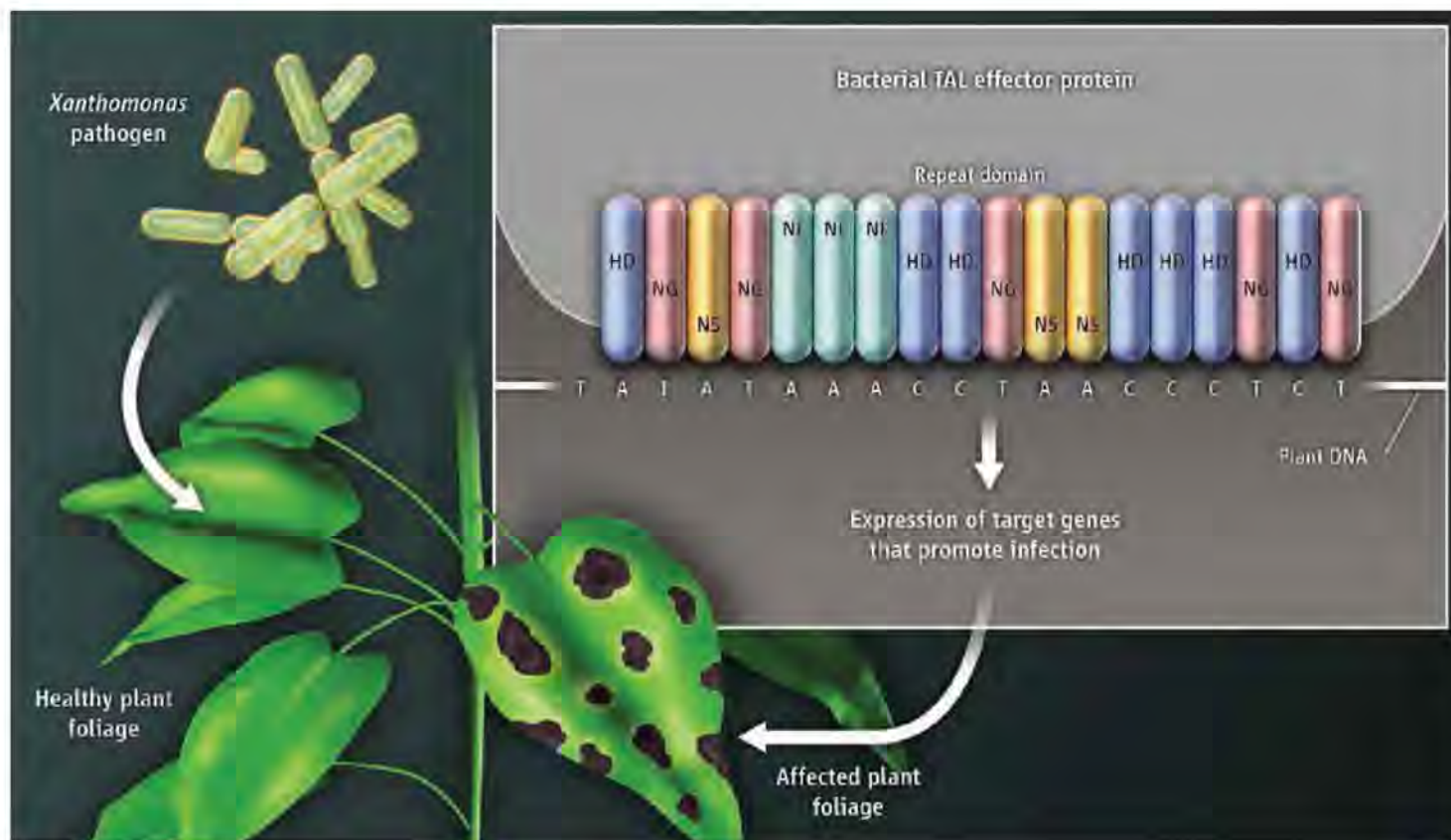
The new motif plays a central role in the function of transcription activator–like (TAL) effectors, proteins expressed by bacterial plant pathogens of the genus *Xanthomonas*. TAL effectors are an important weapon in a battle waged between *Xanthomonas* species and their plant hosts. They are translated in bacteria and deposited into plant cells by the bacteria's type III secretion system. Once in a plant, TAL effectors activate the transcription of plant genes that enable pathogen spread. For example, PthXo1, a TAL effector of a *Xanthomonas* rice pathogen, activates expression of the rice gene *Os8N3*, allowing *Xanthomonas* to colonize rice plants (3). Because *Os8N3* is also necessary for normal plant development, the gene cannot simply be discarded to avoid infection. Rather, there is strong selective pressure for the plant to accumulate mutations that prevent PthXo1 binding, and, consequently, for compensatory changes in the TAL effector's DNA specificity. In the end, DNA recognition determines the outcome of the pathogen–plant war, and so TAL effectors would benefit from a simple, malleable mechanism for DNA recognition. Such a mechanism is revealed by Boch *et al.* and Moscou and Bogdanove in complementary studies that have deciphered the TAL effectors' DNA recognition code.

A mechanism by which proteins of bacterial plant pathogens recognize and control the expression of host plant genes to promote infection is identified.

Both groups focused on the TAL effector's central domain, which contains a variable number of tandem, 34–amino acid repeats (see the figure). The repeat domain was previously shown to bind specific DNA sequences in promoter regions of target genes (4). Amino acid sequences of the repeats are conserved, except for two adjacent highly variable residues (at positions 12 and 13) that were obvious candidates for specificity determinants. Both groups deduced a simple code relating specific diamino acids in the repeat unit to specific nucleotides in the DNA target. Remarkably, there appears to be a one-to-one correspondence between sequential amino acid repeats in the array and sequential nucleotides in the target DNA.

Moscou and Bogdanove broke the DNA recognition code computationally, by searching for nonrandom alignments between the variable diamino acids in the TAL effector and DNA sequences of target promoters. For a handful of well-characterized TAL effectors, DNA sequences identified from the alignments were important for transcriptional activation, thus providing evidence that the alignments and the resulting code were biologically meaningful. Boch *et al.*, on the other hand, deduced the code through molecular genetic analyses of the interaction between AvrBs3 (the TAL effec-

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Code is broken. The DNA binding domain of bacterial TAL effectors contains tandem repeats (about 17; shown in blue, pink, yellow, and green for AvrBs3) of amino acids (34 or 35). The repeat is highly conserved, except for residues at positions 12

and 13 (shown within each repeat; H, histidine; D, aspartic acid; N, asparagine; G, glycine; I, isoleucine; S, serine). The code relates diamino acids in the repeat unit to nucleotides (T, thymine; A, adenine; C, cytosine) in the DNA target.

tor of a *Xanthomonas* pathogen of pepper) and its target DNA sequence in the promoter of an AvrBs3-regulated pepper gene. The deduced code was experimentally validated by several approaches: Target DNA sites were predicted for uncharacterized TAL effectors and shown to confer TAL effector-dependent expression of reporter genes; target DNA sequences were mutated to demonstrate the specificity of TAL effector repeats for predicted nucleotide signatures; TAL effectors expressed in plant cells (heterologous hosts) were shown to activate expression of genes with the predicted target sequences in their promoters; and, quite remarkably, artificial transcription factors with novel DNA binding specificities were created by shuffling the repeat domains and demonstrating their ability to promote the transcription of target genes.

Although both studies establish a central role for the diamino acid sequences in the TAL effectors for recognizing DNA, many important additional questions remain. For example, a three-dimensional structure of a TAL effector complexed to its target DNA may reveal how the diamino acids recognize individual nucleotides within the context of the larger protein domain. Structural information may also clarify whether DNA recognition is influenced by other residues in the effector protein that can-

not be discerned from the primary amino acid sequence of the TAL repeat unit. Apparently, TAL effectors function as dimers (5), making it further difficult to envision how DNA recognition is achieved.

An additional surprising aspect is the modularity of the TAL effector repeat. Boch *et al.* could create novel DNA binding proteins with remarkable ease for a small number of target DNA sites. Similarly, Moscou and Bogdanov's computational analyses suggest that nucleotide specificity is independent of the association of a repeat unit with neighboring repeat units in the TAL central domain. Nucleotide specificity also has some interesting limitations. It is curious that guanine is not recognized by TAL effectors, particularly because guanines are bound with high affinity by other DNA binding motifs (zinc fingers) (6).

Deciphering the TAL effector DNA recognition code suggests several obvious potential biotechnological applications. Most evident is the parallel to artificial zinc finger transcription factors, one of which is currently being evaluated in human clinical trials as a therapeutic agent for diabetic neuropathy (7). Boch *et al.* show that artificial TAL effectors activate transcription in plants; moreover, native TAL effectors activate transcription in yeast (8), suggesting that they may be broadly

useful. The latest rage in genome engineering is to use nucleases with novel DNA binding specificities to create targeted chromosome breaks that stimulate gene targeting (9, 10). The extent to which the TAL effector DNA binding domains can be used for such purposes remains to be determined. It is curious, however, that *Xanthomonas*' simple solution to DNA binding is not more widely used in biology, and this may be a hint that there are limitations to broader applicability. Then again, necessity is the mother of invention, and the host-pathogen conflict has proven time and again to be a rich source of biological innovation.

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ATMOSPHERIC SCIENCE

A New Look at Aging Aerosols

Meinrat O. Andreae

To chemists, studying the composition of the organic matter in atmospheric aerosols has been frustrating. Despite years of effort and the use of the most sophisticated techniques available, only ~10 to 30% of the particulate organic matter (POM) has been identified as specific compounds (1). Given that POM makes up a major part of the atmospheric aerosol, this implies that typically the composition of about half of the material in atmospheric aerosols cannot be characterized as individual compounds. On page 1525 of this issue, Jimenez *et al.* (2) show that the effects of organic aerosol on atmospheric chemistry and climate can be discerned without knowing every one of its components. They propose a new conceptual framework that reflects the emerging view of organic aerosol as a dynamic component of atmospheric chemistry.

Spectroscopic studies have shown that POM contains numerous types of organic molecules, including hydrocarbons, alcohols, aldehydes, and carboxylic acids (3, 4). The unidentified compounds in POM are thought to be a highly complex mixture of combinations of these molecular structures, resulting from oxidation, condensation, and oligomerization of atmospheric hydrocarbons. Conventional analytical techniques require large samples and sampling times of up to a week, and thus the prevailing view of organic aerosols has been that they are a static mixture of ill-defined "organic matter." Because of the limited information available, organic aerosol was thought to have an uneventful life in the atmosphere, beginning with emission from tailpipes or production from oxidation of organic gases, and ending by deposition as dust or in rain.

The transformational event in this story was the invention and widespread use of the aerosol mass spectrometer (5). With this instrument, aerosols in the size range of ~50 to 1000 nm can be analyzed with a time resolution of 1 min, providing chemical information about inorganic species and organic molecular structural components. This tool has vastly increased the information available on the organic component of the atmospheric aerosol (2, 6, 7) and made it possible to relate

changes in the composition, size, and amount of the organic components to chemical and physical processes in the atmosphere.

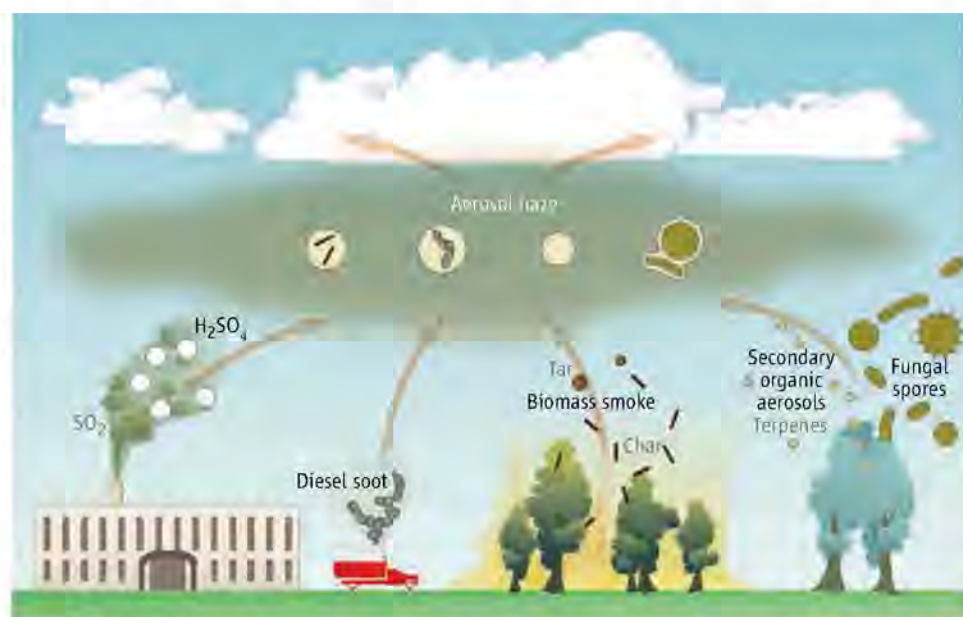
Identification of individual organic species remains an unattainable, but probably not so important, goal. Instead, the aerosol mass spectrometer provides information about chemical properties such as the ratio of oxygen to carbon, the relative abundance of hydrocarbon-like structures, and a variety of molecular fragments. This information allows POM to be classified into a small number of categories that relate to their oxidation state and volatility. This, in turn, provides clues about the origin and processing history of the organic aerosol.

A key concept to emerge is that the organic aerosol is a continuum in a complex space of variables, such as chemical reactivity, degree of oxidation, molecular weight, light absorption, and hygroscopicity (the ability to take up water as the ambient relative humidity rises). Volatility, for example, spans from gaseous compounds, such as isoprene, to humic acid-like substances that resist heating to several hundred degrees (8). Light absorption ability ranges from completely colorless substances to utterly black organic matter (9). These variables are crucial for the ways in which organic

As organic aerosols from different sources age in the atmosphere, their properties become remarkably similar.

matter behaves in the atmosphere and how it affects climate.

Volatility regulates whether a compound is present as a vapor or as condensed matter. This in turn determines whether a substance is more likely to be subject to chemical transformations by gas- or condensed-phase reactions. A large fraction of the atmospheric organic matter is semivolatile and thus readily exchanges between gas and aerosol, allowing a highly dynamic sequence of processes. For example, a compound may move into the gas phase, where it is subject to oxidation reactions by ozone or the hydroxyl radical (OH), followed by recondensation of the less volatile oxidation product, and so on (10). These reaction chains may begin with a molecule of intermediate molecular weight, such as a terpene, which may then undergo oxidation or oligomerization reactions leading to larger molecules, or fragmentation reactions that produce smaller molecules, with CO₂ as the end product. Given the wide variety of starting compounds (both natural and of human origin) that are emitted into the atmosphere, and the complex reaction paths possible in the gas and condensed phases, a myriad of compounds can be formed, with little hope of ever identifying each individually.



Effects of aging. Organic aerosols from different sources undergo chemical processing and mix with each other as well as with inorganic aerosols. This leads to a convergence in their chemical and optical properties and in their ability to nucleate cloud droplets.

Fortunately, such identification may not be necessary, or even particularly helpful, for understanding the role of organic aerosols in atmospheric chemistry and climate, because some of the key variables mentioned above are interrelated and can be derived from properties that can be measured relatively easily. Hygroscopicity is an interesting example: It determines how readily a particle can take up water in a nascent cloud and thus nucleate a cloud droplet, an important way in which aerosols influence climate (11). Jimenez *et al.* show that the hygroscopicity of POM increases in close correlation with its degree of oxidation as measured by aerosol mass spectrometry, indicating that the cloud-nucleating ability of organic aerosols increases as a result of atmospheric chemical processing. Similarly, light absorption by POM increases during aging as a result of the formation of "brown carbon" (12), which affects climate and atmospheric chemistry by absorbing solar radiation.

A remarkable consequence of the atmospheric evolution of POM is the convergence of chemical and physical properties in aerosols of very diverse origin as they age (see the figure). Organic matter produced from pollutants in urban atmospheres, from biogenic emissions in forests, or from terpenes exposed to photochemical reactions in smog chambers all progress to POM of similar oxidation state, hygroscopicity, volatility, and molecular mass. The dynamic exchange of semivolatile substances facilitates the exchange of organic molecules between particles of different origin, promoting, for example, the coating of soot carbon particles with soluble organic matter.

These observations render almost obsolete the classical distinction between primary and secondary atmospheric particles—that is, those emitted as particles and those formed in the atmosphere. They also help to explain the striking similarity in the cloud-nucleating ability of particles from such diverse locations as Mexico City and the remote Amazon rainforest (13, 14). This convergence is a

great advantage to climate modelers, because it reduces the amount of complexity that must be represented in models that investigate aerosol effects on climate.

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MEDICINE

A Reinnervating MicroRNA

Robert H. Brown

MicroRNAs are small noncoding RNAs (~22 nucleotides in length) that negatively regulate gene expression at the posttranscriptional level by binding to the 3'-untranslated region of target RNAs. Over the past 15 years, critical roles for microRNA have been established in regulating cell proliferation, differentiation and development, and death (1). Recent investigations have implicated microRNA networks in diverse disorders, such as cancer and cardiac disease. On page 1549 in this issue, Williams *et al.* (2) define a role for a microRNA (miR-206) in reinnervating the neuromuscular junction after injury and improving survival in a mouse model of the neurodegenerative disease amyotrophic lateral sclerosis (ALS). The findings provide insight into the molecular basis of neuromuscular junction plasticity in the adult and also into determinants of rates of disease progression in motor neuron disease.

Motivated by the observation that microRNAs mediate stress responses in muscle, Williams *et al.* sought evidence that micro-

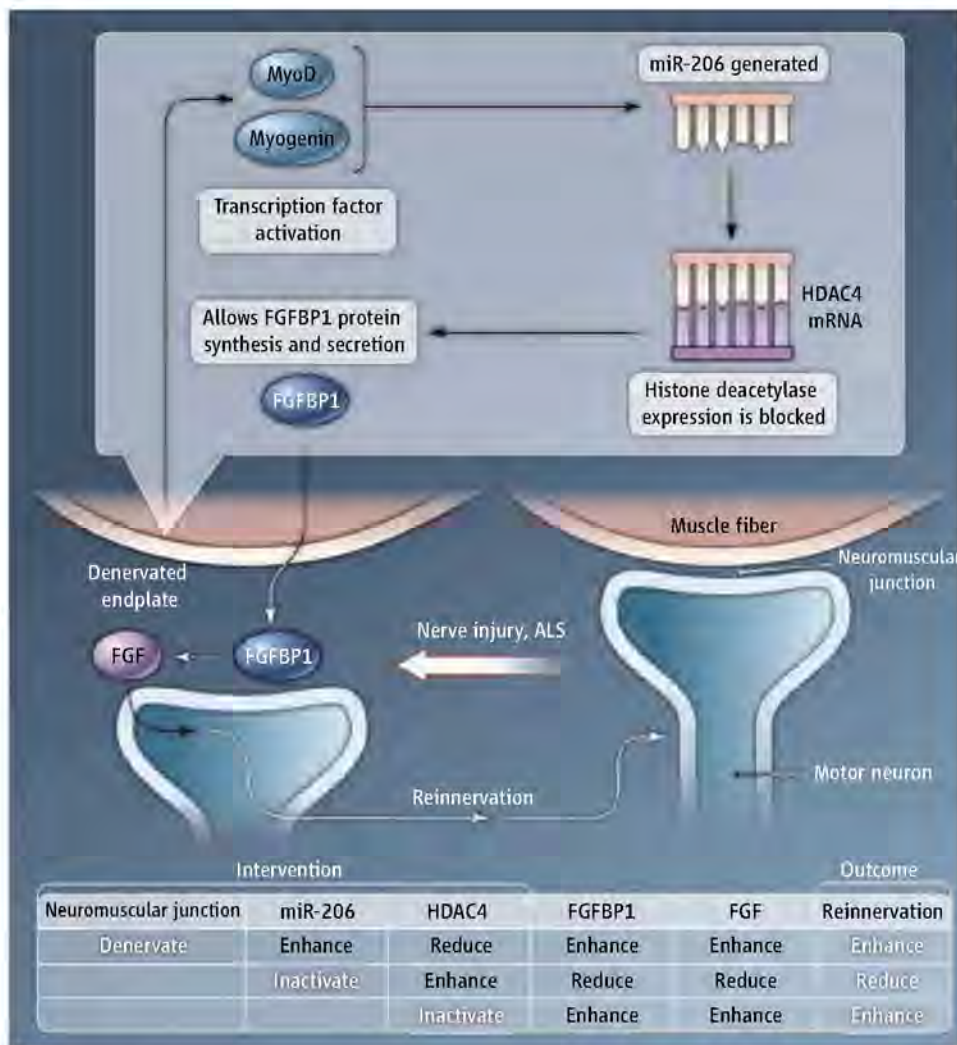
RNAs are dysregulated in the muscle of a transgenic line of the ALS mouse model. In these transgenic ALS mice, a mutant form of the enzyme superoxide dismutase protein (SOD1) was expressed, which triggers adult-onset motor neuron death. In normal mice, sciatic nerve injury, which results in denervation of muscle, increases expression of miR-206 in the muscle. When miR-206 was inactivated in ALS mice, the time from disease onset to death was shortened, indicating that miR-206 is protective in ALS. Similarly, inactivation of miR-206 in normal adult mice adversely influenced the response of the neuromuscular junction to nerve injury. In the absence of miR-206, axonal regrowth toward the denervated neuromuscular junction was normal, but innervation of the junctional endplates (the muscle fiber membrane at the junction between muscles and nerves) was delayed. Moreover, the neuromuscular junctions that did form were morphologically defective. Remarkably, loss of miR-206 had no impact on the normal development of the neuromuscular junctions in uninjured embryos and young mice, indicating a relatively specific role for miR-206 in junction maintenance and regeneration in the adult.

A microRNA expressed in skeletal muscle promotes the regeneration of neuromuscular synapses after injury and in a neurodegenerative disease model.

Williams *et al.* further documented that miR-206 represses the expression of histone deacetylase 4 (HDAC4) in muscle cells. HDAC4 normally inhibits nerve reinnervation by blocking expression of fibroblast growth factor binding protein 1 (FGFBP1). FGFBP1 is thought to potentiate the actions of FGF proteins (FGF7, 10, and 22) on distal motor neuron terminals and thereby promote innervation. Thus, there is a reciprocal interaction between miR-206 and HDAC4; the former facilitates and the latter inhibits reinnervation (see the figure).

These findings are of considerable interest from several perspectives. It is evident that the miR-206–HDAC4–FGFBP1 signaling pathway is important in maintaining the integrity and plasticity of the adult neuromuscular junction. The importance of microRNA function during normal development of the brain is well established. In the nervous system, for example, inactivation of mechanisms that generate microRNA severely disrupts brain structure (3, 4). Less well defined is a role for microRNA in maintaining homeostasis at the neuromuscular junction in the mature nervous system. Williams *et al.* convincingly show that, although it is not essential for normal

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From injury to repair. A damaged (adult) neuromuscular junction triggers a signaling cascade in the muscle fiber that involves the microRNA miR-206. This increases the effect of the growth factor FGF on the neuron, and promotes reinnervation of the muscle. Reinnervation can be enhanced or diminished by altering upstream events.

development of the neuromuscular junction, miR-206 reactivates a developmental pathway (FGFBP1-FGF signaling) to enhance neural repair.

The findings of Williams *et al.* support a growing body of literature documenting that variants in microRNA and their binding sites modify neurological function and influence disease susceptibility. A remarkable example is the report that in Belgian Texel sheep, a single base pair transition in the 3'-untranslated region of messenger RNA (mRNA) encoding myostatin creates a novel binding site for miR-206 and miR-1, another microRNA that is prominently expressed in skeletal muscle (5). The resulting aberrant binding of miR-206 and miR-1 silences the myostatin gene and causes extensive muscle hypertrophy. Another example involves a DNA sequence variation (polymorphism) that is substantially overrepresented in Parkinson's disease and is located in the 3'-untranslated region of

the mRNA encoding FGF20. This variant is insensitive to the microRNA, thus heightening susceptibility to Parkinson's disease by increasing FGF20 expression in the brain. As a consequence, brain α -synuclein accumulates, which can aggregate to form insoluble amyloid fibrils in pathological conditions such as Parkinson's disease (6). MicroRNA networks have also been implicated in other neurodegenerative diseases, including Alzheimer's disease (7), spinocerebellar ataxia (8), and Huntington's disease (9).

A third implication of the study by Williams *et al.* is that it encourages the screening of both microRNAs and their target binding sites for DNA variants that influence the pathogenesis of a wide array of disorders. A challenge in the design of such studies is that only about 10% of precursor microRNAs have polymorphisms; single nucleotide polymorphisms (SNPs) occur at a lower density in microRNAs than in most

of the human genomic DNA (10). Moreover, there is almost no variation in the microRNA "seed" region that is critical for binding to targets. These observations likely reflect evolutionary pressures against variation in microRNA and target sequences, and are consistent with the thesis that mutations in microRNA can be pathogenic. Given the paucity of SNPs in microRNA and microRNA targets, screening for pathological variants by SNP analysis is likely to be much less effective than direct sequencing. Fortunately, deep sequencing methods now make it feasible to perform in-depth resequencing of microRNAs, their predicted targets, and related genes (11).

As illustrated in the present study of miR-206, pathways delineated by analyses of microRNA pathology provide targets for therapy. Interventions to augment the sprouting of neuron terminals and their innervation of muscle might include small-molecule mimetics of miR-206 or perhaps miR-206 itself, agents that block repression of FGFBP1 by HDAC4, or inhibitors of HDAC4. It is intriguing in this context that HDAC inhibition is already in clinical trial for spinal muscular atrophy, another motor neuron disease (12).

As the concept of the miR-206-HDAC4-FGFBP1 signaling pathway evolves, numerous questions remain. The relative importance of myogenin and MyoD as regulators of miR-206 expression and activity remains unclear. It is also unknown if the degree of innervation and synaptic activity influence the miR-206 signal. And which FGF receptor types respond to FGFBP1, and how do they signal to the distal motor neuron terminal to sprout? Such information will further delineate the biological properties of the synaptic junction and the repertoire of responses of mature neuromuscular junction to injury, thereby providing additional insights into possible new avenues for treating disorders like ALS.

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Lumpers and Splitters: Darwin, Hooker, and the Search for Order

Jim Endersby

Classification was a key practice of the natural history sciences in the early 19th century, but leading taxonomists disagreed over basic matters, such as how many species the British flora contained. In this arena, the impact of Charles Darwin's ideas was surprisingly limited. For taxonomists like Darwin's friend, Joseph Dalton Hooker, the priority was to establish a reputation as a philosophical naturalist, and to do so Hooker embarked on a survey of global vegetation patterns. He believed that taxonomic "splitters" hindered his ambition to create natural laws for botany (and hence establish it as a prestigious science) by generating a multitude of redundant synonyms for every plant variety. Despite the fact that Darwin's ideas apparently promised a justification for splitting, they also offered a philosophical justification for Hooker's taxonomic practice, and so he enthusiastically championed his friend.

The claim is often made that Darwin changed the world in part because *On the Origin of Species* shattered people's religious faith and ushered in a period of turmoil. There are many reasons to be skeptical about this interpretation, not least that many of *Origin's* readers regarded it as neither creating nor exacerbating a "war" between science and religion (1–3). Yet even if reports of religious war are exaggerated, surely there can be no doubt about Darwin's revolutionary impact on science? However, for some of his most important scientific allies, Darwin's ideas promised a robust justification for conducting scientific business as usual, and I want to argue that Darwin's "revolution" was actually profoundly conservative (4–6).

The impact of Darwinism on Victorian science is clearly reflected in the career of his closest friend, the botanist Joseph Dalton Hooker (Fig. 1). Hooker was the son of William Jackson Hooker, Regius Professor of Botany at Glasgow University. This prestigious-sounding title could not conceal the fact that the botany professor earned a modest living by teaching those whom Joseph Hooker referred to as "that lowest of all classes of students, the medical" [quoted in (7)]. Medicine was regarded as a lowly trade, in which cures were rare and scientific understanding of disease even rarer, and, because the few paid positions for botanists involved teaching trainee medical men, botany's scientific standing suffered from the association. Moreover, medical botany was typical of the uses of 19th-century botany, which were mainly practical or commercial; such useful sciences were frequently considered to be of lower status than elite studies, such as mathematics and physics.

A Naval Botanist

Hooker began his career with a long ocean voyage, spending 4 years exploring the Antarctic aboard

HMS *Erebus*, which was mapping Earth's magnetic field (Fig. 2). This was a common path for putative men of science to take, and of course Darwin's scientific life had also begun aboard a ship. Darwin had traveled in style as a gentleman companion to the *Beagle's* captain, whereas Hooker was a badly paid junior naval surgeon whose father, unlike Darwin's, did not have a fortune to bequeath him. During the voyage, William Hooker became director of the Royal Botanic Gardens at Kew, which helped him add to the network of influential friends who helped him secure his son

half-pay from the Admiralty while he wrote up the results of his expedition. Yet despite this assistance, Joseph Hooker spent over a decade searching for a scientific position that was both better remunerated and more prestigious than teaching medical students.

As the ship sailed homeward, Hooker analyzed his future prospects in a letter to his father, noting that "I am not independent, and must not be too proud; if I cannot be a naturalist with a fortune, I must not be too vain to take honourable compensation for my trouble" [quoted in (7)]. Clearly being "a naturalist with a fortune" would have been preferable to accepting money. His statement reflects the fact that, for much of the 19th century, the ideal man of science was someone like Sir Joseph Banks, an independently wealthy gentleman who put his money and expertise at the nation's service and accepted no financial reward, his wealth being a guarantee of his disinterestedness (i.e., having no financial stake allowed him to pursue truth without thought of personal gain). Victorian men of science like Hooker struggled to retain some of the status associated with the old gentlemanly ideal while nevertheless demanding that their merits be recognized and properly rewarded. One result of this tension was that for the emerging class of professional scientists the highest accolade was to be described not as a professional, but as a "philosophical" naturalist. In this context, "philosophical" was a word with many complex meanings, starting with its derivation from the term "natural philosophy," which encompassed



Fig. 1. Joseph Hooker at his desk, microscope in hand. From the original by T. B. Wigram (published 17 July 1886 in *The Graphic*, p. 64), held at Kew. [Copyright, The Board of Trustees of the Royal Botanic Gardens, Kew]

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Fig. 2. The *Erebus* and *Terror* at Kerguelen's Land. [Reprinted from (23)]

branches of science that sought to understand and explain the causes of natural phenomena. By contrast, natural history was merely descriptive: cataloging and naming, but not explaining. Natural philosophy was the forerunner of the elite sciences, especially physics, which provided the model for those naturalists who wished and worked to raise their disciplines to comparable status.

In 1833, the *Edinburgh Review* noted that a field like botany had traditionally been undervalued and that its position appeared to be gradually changing, thanks to the appearance of "the philosophical botanist, who invents new principles of classification, who studies the structure and organs of plants, who develops the laws of their geographical distribution, and who investigates their uses in relation to diet, medicine, and the arts" (8). The reviewer's comments were typical of discussions within the botanical community at the time and provided an implicit definition of what it meant to be philosophical for men like Hooker who were working to develop "new principles of classification," study the "structure and organs of plants" (plant anatomy and physiology), and, above all, develop the laws governing plants' "geographical distribution."

The Laws of Botany

For a botanist like Hooker, there were two main attractions to understanding the complex patterns of vegetation. First, imposing laws on botany would elevate it from the merely descriptive. Second, unraveling the mysteries of plant distribution had a more practical, economic benefit at a time when much of the wealth of Britain's empire rested on plants: from the timber and hemp from which her navy was built to the indigo, spices, opium, tea,

cotton, and thousands of other plant-based products that the ships carried (Fig. 3). Understanding the laws that shaped vegetation allowed valuable new plants to be discovered and allowed existing crops to be successfully transplanted to British colonies, where they could be cultivated profitably. A grateful nation might reasonably be expected to reward the science that had added to the empire's wealth. Hence, unsurprisingly, Hooker described the "great problems of distribution and variation" as "prominent branches of inquiry with every philosophical naturalist" (9).

However, before the philosophical naturalist could pursue the study of distribution, classifications needed to be resolved. Hooker was convinced that many of the plants named as species were merely varieties, which had been elevated to specific status by those he called splitters. Abolishing names that he considered invalid would simplify the maintenance of Kew's rapidly expanding collection of dried plant specimens (herbarium), where every species name required a separate specimen on a separate sheet. More importantly, the key tool Hooker and his colleagues used to analyze distribution was known as botanical arithmetic, the calculation of the precise ratio of species to genera at each locality that they hoped would give numerical precision to previously vague statements about different regions being rich or poor in species. Such calculations could not be done until every botanist agreed on common definitions of species and genus and classified according to common principles.

Hooker's desire to grasp the laws of plant distribution helps to explain his hostility to the inexperienced colonial botanists, of whom he wrote that, whenever they found a plant they

could not name, "it rarely enters into his head to hesitate before proposing a new species." The result was the creation of duplicate names, synonyms, which he described as "the greatest obstacle to the progress of systematic botany" (10).

A Colonial Naturalist

Among the most avid namers of new species was William Colenso (Fig. 4), a missionary naturalist in New Zealand who collected plants for Hooker over many years. On the one hand, Hooker valued his correspondent's work so highly that he dedicated his flora of New Zealand to Colenso and a fellow colonial naturalist (11). On the other hand, Hooker was adamant that Colenso should not attempt to name his adopted country's plants himself but cede that right to metropolitan experts like himself. Hooker concluded his New Zealand flora with a lengthy essay on the principles of botany, in which

he addressed those like Colenso whom he knew would disagree with some of his classifications. He wrote that, "the New Zealand student will at first find it difficult to agree with me in many cases, as for example on so protean a Fern as



Fig. 3. Economic botany. A sample of rubber collected by Hooker during his Indian travels. [Copyright, The Board of the Trustees of the Royal Botanic Gardens, Kew]

Lomaria procera, whose varieties (to an inexperienced eye) are more dissimilar than are other species of the same genus" (12). This might almost have been addressed to Colenso personally, because he was a specialist on ferns and repeatedly attempted to name them; his unpublished botanical notebooks list more than a dozen specific names for the plant that Hooker called *Lomaria procera* (now *Blechnum procera*). Colenso believed his detailed, first-hand knowledge of New Zealand's plants gave him a greater expertise than Hooker, who had spent only 3 months in the country. However, Hooker was equally convinced that the herbarium gave him the final word because he was able to compare New Zealand's plants with examples from around the world. If this were done, Hooker argued, it became clear that the apparently distinct New Zealand forms could be placed within an unbroken sequence of similar forms, and so, Hooker firmly asserted, the plant "ranks according to my philosophy as a variety and not as a species" [quoted in (7); see also (13)].

The fact that varieties that were distinct in some locations disappeared in others was, in Hooker's view, proof that "no deduction drawn from local observations on widely distributed plants can be considered conclusive." He added that

To the amateur these questions are perhaps of very trifling importance, but they are of great moment to the naturalist who regards accurately-defined floras as the means of investigating the great phenomena of vegetation (12, p. xiv).

From Hooker's perspective, the potential for confusion created by the "splitters" was a major obstacle to his wider project to raise the status of botany by globally mapping vegetation types, hence the need to persuade the colonials to toe the imperial line.

Disagreement as Evidence

Hooker, therefore, was a "lumper," one who defines species broadly, submerging many minor varieties under a single name, whereas a splitter does the opposite, naming the varieties as subspecies or even full species. From Darwin's perspective, each had their uses, as he told Hooker in 1857, "it is good to have hair-splitters and lumpers" (14). Hooker would undoubtedly have demurred, but for Darwin the disagreements between lumpers and splitters were evidence for evolution. He referred in *Origin* to those genera that were so hard to classify that "hardly two naturalists can agree which forms to rank as species and which as varieties," giving as instances *Rubus* (brambles) and *Hieracium* (hawkweeds). These plants were notoriously difficult to name and classify, and experts clashed regularly over how many species there were in each group (15). For Darwin, these "polymorphous" genera were evidence of evolution in progress because they were precisely what his theory would predict: If every species had been created in its modern

form, their boundaries should be clearly defined; but, if each species evolved from another, there ought to be cases where the random variations that characterized all living things had yet to be sifted by natural selection or where extinction had not yet created the gaps that allowed species to be clearly discerned and named. Darwin therefore argued that "a well-marked variety may be justly called an incipient species," [p. 57 of (19)] and thus all those varieties of hawkweed or bramble were species in the making. From Hooker's perspective, the thought of species being constantly formed was a nightmare. There were, he argued, already far too many so-called species in existence, most of them named by provincial and colonial splitters.



Fig. 4. The Reverend William Colenso in old age. [Copyright, The Board of Trustees of the Royal Botanic Gardens, Kew]

Although a well-connected metropolitan expert like Hooker might be assumed to be able to impose his views on a colonial naturalist like Colenso, the prevailing disagreements between British taxonomists undermined his authority. Between 1858 and 1862 three major British floras appeared, all of which used what was supposedly the same taxonomic system. Yet an anonymous reviewer noted that each came to wildly different conclusions as to how many plant species there were in Britain. The reviewer noted that "While Mr. Babington's Manual (Ed[ition]. iv) contains 1708, Messrs. Hooker and Arnott have but 1571, and Mr. Bentham 1285"; in other words, Bentham's contained only three-quarters of the number of species found in Babington's book (16). How could botany be considered a mature science if leading botanists could not agree on the simple issue of how many species there were in a well-botanized locality like Britain? Colenso was well aware of these disputes, telling Hooker that

To the question,—*What constitutes a really distinct genus, or species?* I cannot give a satisfactory answer. I know not of any certain rule; and I find the first Botanists of the day opposing one another in their speculations; while not a few are laboriously undoing what their predecessors or compeers have toiled to rear (17).

Given these disputes, Colenso could see no reason not to enter the fray himself, especially as he knew the living plants of his country better than anyone who relied entirely on dried specimens.

Supporting Darwin

Hooker was the first man of science to publicly endorse Darwin's theory. Within a few weeks of *Origin's* first publication, Hooker published his essay "On the flora of Australia," in which he announced his support for "the ingenious and original reasonings and theories by Mr. Darwin and Mr. Wallace" (9). One year earlier, after Darwin's and Alfred Russel Wallace's papers had first been read at the Linnean Society of London, Hooker had written to the American botanist Asa Gray that he was "most thankful"

that I can now use Darwin's doctrines — hitherto they have been kept secrets I was bound in honor to know, to keep, to discuss with him in private — but never to allude to in public, & I had always in my writings to discuss the subjects of creation, variation &c &c as if I had never heard of Natural Selection — which I have all along known [quoted in (18, pp. 32–33)].

Hooker's pleasure at being able to make use of Darwin's ideas suggest excitement that a long-standing problem was being solved, a new era in natural history was being inaugurated. However, this was only partly true. Darwin had claimed in *Origin* that "When the views entertained in this volume on the origin of species, or when analogous views are generally admitted, we can dimly foresee that there will be a considerable revolution in natural history," and yet he went on to say that despite his revolution, "systematists will be able to pursue their labors as at present" (19). Hooker understood this argument very well (not least because it had been shaped by the discussions he and Darwin had had over the previous 15 years). In his essay, Hooker argued that "the descriptive naturalist who believes all species to be derivative and mutable [the evolutionist], only differs in practice from him who asserts the contrary, in expecting that the posterity of the organisms he describes as species may, at some indefinitely distant period of time, require re-description." For all practical purposes, there was no difference between post- and pre-Darwinian taxonomy: "the believer in species being lineally related forms must employ the same methods of investigation and follow the same principles

that guide the believer in their being actual creations" (9).

A scientific revolution that makes no difference to everyday scientific work seems an odd sort of revolution, yet it was precisely this conservatism that helped make Darwin's version of evolution acceptable to naturalists who had rejected earlier theories.

Evolving Philosophy

Hooker's intense dislike of splitters becomes more understandable in the context of his career and the grand imperial project that made it possible. Yet we are still left wondering why he was the first British man of science to publicly embrace Darwin's theory of evolution, because he must have foreseen that Darwin's claims that varieties were in fact incipient species would be gleefully seized on by the splitters. Colenso, for example, became an ardent Darwinist, and although no record survives of him using Darwin's name to justify giving specific names to varieties, other naturalists certainly did.

If, as Hooker implied, Darwinism required no change to day-to-day scientific practice, why embrace his friend's theory at all? Loyalty was only part of the reason; to fully understand, we need to look again at what Darwin said about classification in *Origin*.

After analyzing the principles of classification, especially the difficult cases like the hawkweeds and brambles, Darwin concluded that

All the foregoing rules and aids and difficulties in classification are explained, if I do not greatly deceive myself, on the view that the natural system [of classification] is founded on descent with modification; that the characters which naturalists consider as showing true affinity between any two or more species, are those which have been inherited from a common parent, and, in so far, all true classification is genealogical. (19, pp. 324–325)

If Darwin was right, classification was much more than mere naming; it was uncovering the history of life on Earth—a history that, when combined with the insights of geology, also explained much about the distribution of plants across the globe. Evolution provided the life sciences with laws, explanations for the patterns that had previously been recorded but not explained. That, for Hooker, was the great attraction of evolutionary ideas; they provided a fully philosophical underpinning for his work, a firm basis from which to tell the splitters he was right and they were wrong. The broadly defined species that Hooker used were, he argued, all the descendants of a common ancestor. He argued that if

we consider these closely allied varieties and species as derived by variation and natural selection from one parent form at a comparatively modern epoch, we may with

advantage, for certain purposes, regard the aggregate distribution of the very closely allied species as that of one plant (20, p. 279).

This was lumping with a vengeance, but not on the basis of an idiosyncratic whim; it was justified by Darwin's theories. As he had argued 2 years earlier, Darwin's ideas "should lead us to more philosophical conceptions on these subjects, and stimulate us to seek for such combinations of their characters as may enable us to classify them better, and to trace their origin back to an epoch anterior to that of their present appearance and condition" (9).

In *Origin*, Darwin had promised systematists that their "shadowy doubt" about whether or not a particular type was really a species would cease and was confident, having spent 8 years of his life classifying barnacles, that this "will be no slight relief." Instead, they will simply need to assess "whether any form be sufficiently constant and distinct from other forms, to be capable of definition" (19). The prospect of objective classifications that would put an end to rancorous disputes between taxonomists, combined with providing natural history with some proper scientific laws, were the major attractions of the Darwinian theory to men like Hooker. Nevertheless, Hooker's concern to bring stability to classification also explains his apparently contradictory statements about the impact of evolution. Not only should pro- and anti-Darwinian botanists "employ the same methods of investigation and follow the same principles," but he went so far as to argue that Darwin's "hypotheses should *not* influence our treatment of species, either as subjects of descriptive science, or as the means of investigating the phenomena of the succession of organic forms in time, or their dispersion and replacement in area" (9).

Although reining in the splitters remained an urgent task and despite the advantage it gave to splitters, the philosophical attractions of evolution by natural selection help explain why Hooker became and remained a staunch and loyal defender of Darwin. Hooker embraced the mechanism of natural selection more enthusiastically than many of his contemporaries, speaking out publicly in support of his friend's ideas.

Revolutionary Failure

The terms lumpers and splitters are still very much in use among taxonomists and applied, usually pejoratively, to those whose view of the limits of species differs from the writer's own. The fact that they are still in use marks the failure of at least one aspect of the Darwinian revolution: In *Origin*, Darwin had assured his readers that "Our classifications will come to be, as far as they can be so made, genealogies," with the result that "the rules for classifying will no doubt become simpler," once naturalists learned "to discover and trace the many diverging lines of descent in our natural genealogies" (19). Darwin's claim has inspired systematists ever since, but despite its near-

universal acceptance there is still no comparable agreement as to exactly how to put it into practice (21, 22). Hooker's story suggests that there may never be agreement: What worked for an metropolitan naturalist working at the heart of a great empire could never satisfy a colonial naturalist, eager to record the minute details that years of close study had revealed. Somewhat ironically, the work of many of those Hooker condemned as splitters has since proved of more use to modern evolutionary biologists than his broad species, because the splitters recorded evidence that is now useful for the study of speciation, biodiversity, and climate change. The way we classify is ultimately a product of why we classify.

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A Simple Cipher Governs DNA Recognition by TAL Effectors

Matthew J. Moscou and Adam J. Bogdanove*

TAL (transcription activator-like) effectors of plant pathogenic bacteria in the genus *Xanthomonas* contribute to disease or trigger defense by binding host DNA and activating effector-

the 54-base pair (bp) *UPA20* promoter fragment that is sufficient and necessary for activation, and it coincided with the *UPA* box common to genes directly activated by AvrBs3 (3). For effectors PthXo1

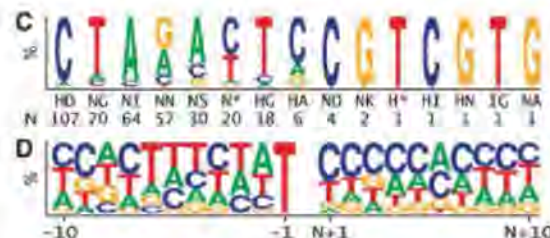


Fig. 1. The TAL effector–DNA recognition cipher. **(A)** A generic TAL effector showing the repeat region (open boxes) and a representative repeat sequence with the RVD underlined. **(B)** Best pattern matches (low-entropy alignments) for several TAL effector RVD and target gene promoter sequences. An asterisk indicates a deletion at residue 13. **(C)** RVD-nucleotide associations in the alignments in (B) and 10 more alignments obtained by scanning all rice promoters with 40 additional *X. oryzae* TAL effectors, retaining for each effector the best alignment for which the downstream gene was activated during infection. **(D)** Flanking nucleotide frequencies for the 20 TAL effector target sites. Positions are relative to the 5' end of the target site; *N*, length of target site. Logos were generated using WebLogo (<http://weblogo.berkeley.edu/>).

specific host genes (1–5). Specificity depends on a variable number of imperfect, typically 34, amino acid repeats (6). Polymorphism is primarily at repeat positions 12 and 13, which we call the repeat-variable diresidue (RVD). We show that the RVDs of TAL effectors correspond directly to the nucleotides in their target sites, one RVD to one nucleotide, with some degeneracy and no apparent context dependence. Our finding explains TAL effector specificity, enables target site prediction, and opens prospects for use of these proteins in research and biotechnology.

Several considerations suggested that RVDs would specify DNA targets. Structural predictions place residues 12 and 13 on a solvent-exposed surface (6). Binding of TAL effector AvrBs3 to the *UPA20* promoter is mediated by its repeats (3). And, the AvrBs3 target *Bs3* is activated also by TAL effector AvrHah1, which despite overall dissimilarity has a similar sequence of RVDs (7). We thus anticipated a one-to-one correspondence between RVDs and contiguous nucleotides in the target site. To test this hypothesis, for each of 10 known TAL effector-target gene promoter pairs, we scanned for RVD-nucleotide alignments with minimal entropy.

Low entropy sites were present in each promoter. However, for AvrBs3, only one mapped to

and AvrXa27, only one site each overlapped a polymorphism between the activated and nonactivated alleles of their respective targets, *Os8N3* and *Xa27*. Across the alignments at these three sites, RVD-nucleotide associations were consistent. Remaining alignments were selected on the basis of those associations, resulting in exactly one site per TAL effector-target pair (Fig. 1). A T precedes each site.

To assess the specificity conferred by the RVD-nucleotide associations, we generated a weight matrix based on their frequencies and scanned about 60,000 annotated promoters in rice (cv. Nipponbare) for best matches to the five TAL effectors from the rice pathogen *X. oryzae*. For four, the experimentally identified target gene was the best or nearly best match. Better matches were not preceded by a T, were not represented on the microarray used to identify the target, or lacked introns and expressed sequence tag evidence. Scanning the reverse complement promoter sequences yielded no better scoring alignments than the forward sites for the known targets. The known target of the fifth effector, AvrXa27, is the disease resistance gene *Xa27* (1). The poorer rank for this match (5368) may reflect a suboptimal or calibrated host adaptation. Better scoring sites likely comprise genes targeted by AvrXa27 for pathogenesis.

We obtained 10 more alignments by scanning all rice promoters with 40 additional *X. oryzae* TAL effectors. We retained the best alignments for which the downstream gene was activated during infection based on public microarray data (<http://PLEXdb.org>, accession OS3). Here too a T precedes each site, and no reverse-strand sites scored better. The RVD-nucleotide alignments constitute a strikingly simple cipher (Fig. 1C).

There is some degeneracy in the cipher. Strong associations may represent binding anchors. Weak ones may provide flexibility or result from neighbor effects. Analysis of the latter, however, yielded no signal, suggesting context independence.

Sequences flanking the 20 target sites tend to be C-rich after the site and G-poor throughout (Fig. 1D). The sites usually begin within 60 bp upstream of the annotated transcriptional start. None are closer than 87 bp to the translational start.

Annotation of TAL effector targets, now feasible, will aid identification of host genes important in disease. Adding TAL sites may enhance efficacy and durability of resistance genes like *Xa27*. TAL effectors may also be useful for targeted gene activation as well. Whether TAL effectors function in nonplant cells or are amenable to protein fusion are unknown. Elucidating their interaction with host transcriptional machinery and their structure bound to DNA are important next steps in defining the function and utility of these proteins.

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Supporting Online Material

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Figs. S1 and S2

Tables S1 and S2

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Cell-Specific Information Processing in Segregating Populations of Eph Receptor Ephrin-Expressing Cells

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Cells have self-organizing properties that control their behavior in complex tissues. Contact between cells expressing either B-type Eph receptors or their transmembrane ephrin ligands initiates bidirectional signals that regulate cell positioning. However, simultaneously investigating how information is processed in two interacting cell types remains a challenge. We implemented a proteomic strategy to systematically determine cell-specific signaling networks underlying EphB2- and ephrin-B1-controlled cell sorting. Quantitative mass spectrometric analysis of mixed populations of EphB2- and ephrin-B1-expressing cells that were labeled with different isotopes revealed cell-specific tyrosine phosphorylation events. Functional associations between these phosphotyrosine signaling networks and cell sorting were established with small interfering RNA screening. Data-driven network modeling revealed that signaling between mixed EphB2- and ephrin-B1-expressing cells is asymmetric and that the distinct cell types use different tyrosine kinases and targets to process signals induced by cell-cell contact. We provide systems- and cell-specific network models of contact-initiated signaling between two distinct cell types.

Signal transduction is typically studied by the stimulation of target cells with a soluble extracellular ligand, such as a growth factor (1). However, this approach does not necessarily recapitulate the physiological process of intercellular communication, in part because protein ligands for some transmembrane receptors may themselves be attached to the surface of cells. Furthermore, signaling initiated by cell-cell contact is typically a reciprocal process, in which two cell types exchange distinct signals, leading to mutually dependent alterations in their respective behaviors. For example, events such as axon guidance, antigen presentation, and generation of apical-basal polarity can involve two or more cells that directly interact through membrane-tethered receptors to exchange signaling information (2). In particular, direct interactions between transmembrane Eph receptor tyrosine kinases (EphRs) and their membrane-bound ephrin ligands frequently lead to mutual cell repulsion and are important for axon guidance and boundary formation during tissue development (3–6).

EphRs, which make up the largest family of mammalian receptor tyrosine kinases, bind cell surface-associated ephrins that have either a glycosylphosphatidylinositol (GPI) membrane

anchor (A-type ephrins) or a transmembrane region followed by a conserved cytoplasmic tail (B-type ephrins). Clustering of B-type EphRs and ephrins at the surface of adjacent cells activates phosphotyrosine (pTyr) signaling in both the EphR- and ephrin-expressing cells, termed forward and reverse signaling, respectively (7–9). Ephrins lack intrinsic catalytic activity, and reverse signaling is achieved through the activation of associated tyrosine kinases and direct binding of intracellular targets (10, 11). Analysis of EphR or ephrin signaling initiated with artificial soluble ligands provides limited understanding of the cell-specific signaling networks (6, 12) because signaling between neighboring EphR- and ephrin-expressing cells may also be affected by inputs from adhesion molecules and the extracellular matrix (6, 13, 14). We therefore set out to identify cell-specific responses, including quantitative differences between the underlying multivariate signaling networks in the two cell types (15, 16).

Systematic analysis of cell-specific networks in distinct populations of interacting cells is challenging primarily because the unique properties of each cell type are lost once cocultured cells are processed for biochemical analysis, such as by immunoblotting. Thus, to investigate bidirectional EphR-ephrin signaling in the context of direct cell-cell interactions we developed a proteomic strategy termed quantitative analysis of Bidirectional Signaling (qBidS), which is based on lineage-specific labeling with stable isotopomeric versions of amino acids (SILAC), specifically arginine and lysine, combined with mass spectrometric identification and relative quantification of tyrosine-phosphorylated peptides. Systematic evaluation of mixed populations of EphR- and ephrin-expressing cells with this

approach was then combined with a phenotypic analysis of EphR- and ephrin-dependent cell sorting by means of small interfering RNA (siRNA) screening and data-driven computational modeling of signaling networks. This identified extensive cell-specific information processing as well as asymmetric network structure and utilization during bidirectional signaling between EphB2- and ephrin-B1-expressing cells.

Quantitative analysis of bidirectional Eph receptor–ephrin signaling. To study bidirectional EphR-ephrin signaling, we used human embryonic kidney (HEK) 293 cell lines engineered to express either EphB2 (EphB2⁺ cells) or ephrin-B1 (ephrin-B1⁺ cells) (figs. S1 and S2) (17, 18). These cells show a repulsive response when they contact one another in cell culture and display self-organizing properties so that the EphB2⁺ cells, which are marked with a membrane-targeted myristoylated green fluorescent protein (GFP), form multicellular colonies with sharp boundaries that exclude ephrin-B1⁺ cells. To quantitatively investigate the response of signaling networks in either EphB2⁺ or ephrin-B1⁺ cells, we used SILAC (19, 20) to differentially label the two cell types.

EphB2⁺ cells were grown independently in the presence of “light” (C12N14) arginine and lysine or with “heavy” (C13N15) arginine and lysine (21). Ephrin-B1⁺ cells were labeled separately with “medium” (C12N15) arginine and lysine (Fig. 1A). The incorporation of these amino acids into the proteins of each cell line enabled relative quantification of signaling events in a cell line-specific manner (Fig. 1, A and B). To initiate signaling by cell-cell contact, medium-labeled ephrin-B1⁺ cells were combined with the adherent heavy-labeled EphB2⁺ cells. After 10 min, the cells were lysed under denaturing conditions and mixed with light-labeled, nonstimulated EphB2⁺ cells as a point of reference (Fig. 1A). Tyrosine-phosphorylated peptides were isolated and analyzed with liquid chromatography-mass spectrometry (LC-MS) on an LTQ-Orbitrap (Thermo Scientific, San Jose, California) (18, 22, 23). The peptide data are available at www.epphomics.org.

Because peptides with identical sequences but originating from distinct cell lines differ by the mass differences resulting from the SILAC labeling, their relative abundance can be determined by comparing their extracted ion currents (Fig. 1B). This enables quantification of protein phosphorylation specific to the EphB2⁺ cells by using the ratio between heavy-labeled peptides (EphB2⁺, stimulated) and the corresponding light-labeled peptides (EphB2⁺, control). At the same time, peptides originating from ephrin-B1⁺ cells that were used for stimulation can be distinguished by their medium labeling (Fig. 1, B and C). As examples, we observed that phosphorylation of the activation loop of EphB2 (Y780) was increased by 80% after mixing EphB2⁺ and ephrin-B1⁺ cells. Conversely, a tyrosine-phosphorylated peptide from the intracellular region of ephrin-B1 (Y317)

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was only observed with the medium label, showing that it specifically originated from the ephrin-B1⁺ cell population. We identified pTyr sites in previously described EphB2 targets, such as the PDZ domain-containing protein Afadin [AF6 (Y1657)] and the adaptor protein SHC1 (Y427), both of which were threefold more abundant in the stimulated EphB2⁺ cell population (Fig. 1C). The experimental approach was then reversed so as to identify dynamic pTyr signaling in ephrin-B1⁺ cells mixed with EphB2⁺ cells (fig. S3) (18).

Bidirectional signaling between EphB2- and ephrin-B1-expressing cells is asymmetric. Analysis of the global changes in tyrosine phosphorylation induced by contact between EphB2⁺ and ephrin-B1⁺ cells identified a total of 442 tyrosine phosphorylation sites on 304 target proteins that significantly increased or decreased in abundance in one or both cell types ($P < 0.05$; Wilcoxon test) (table S1) (18). Contact-initiated signaling between EphB2⁺ and ephrin-B1⁺ cells affected pTyr sites from proteins with a wide range of cellular functions and revealed both known and previously undescribed targets (Fig. 2A and fig. S4). These include regulators of adhesion, polarity, phosphoinositide signaling, actin remodeling, endocytosis, and cell-cell interactions, implicating these cellular processes in the regulation of EphR-ephrin-controlled cell sorting.

To quantitatively assess cell-specific signaling events, and to avoid sampling bias from the MS analysis, we identified identical peptides that were tyrosine-phosphorylated in both EphB2⁺ and ephrin-B1⁺ cells and compared their dynamic profiles between the two cell types. Overall, these profiles revealed a shifted distribution ($P < 1 \times 10^{-36}$; Wilcoxon test) (fig. S5A), confirming

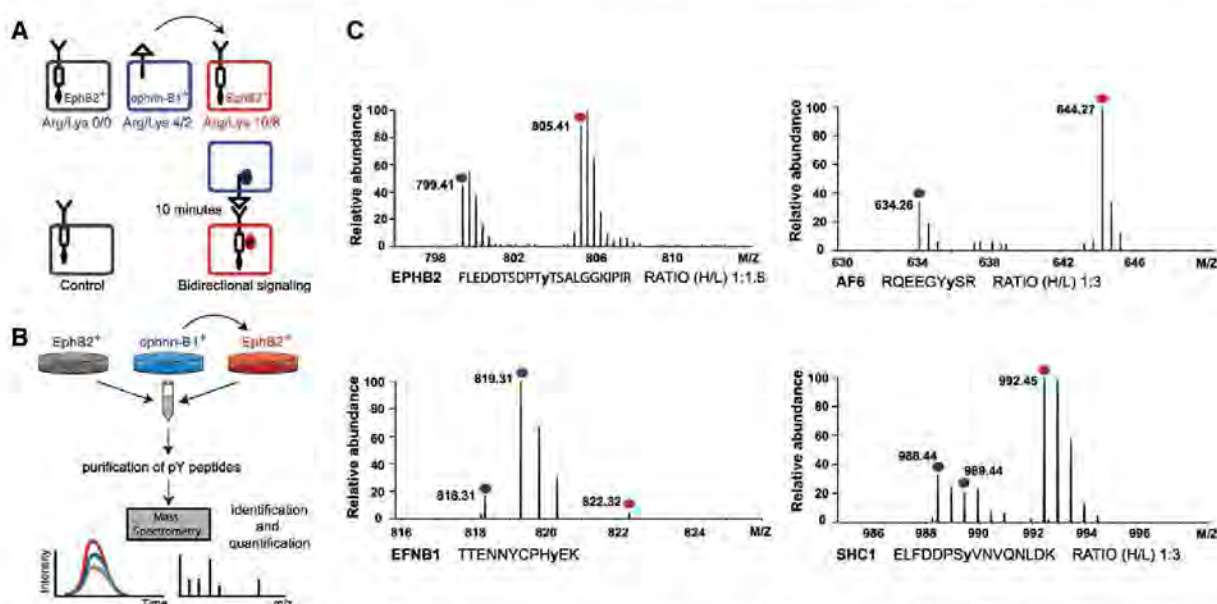
that the responses initiated by cell-cell contact differ between the distinct cell populations. We compared the cell-specific modulation of the 100 common tyrosine phosphorylation sites, which we grouped according to the signaling domains present on the phosphorylated proteins (Fig. 2B). Of these sites, 71% displayed asymmetric modulation between the cell lines ($P < 0.05$; Wilcoxon test). Adaptor proteins such as SHB, SHC1, and DOK1 displayed significantly increased tyrosine phosphorylation in stimulated EphB2⁺ cells, whereas in ephrin-B1⁺ cells the phosphorylation of these sites was decreased ($P < 0.05$; Wilcoxon test). The phosphorylation of focal adhesion kinase [PTK2 (Y576 and Y577)] and paxillin [PXN (Y118)] was increased in both EphB2⁺ and ephrin-B1⁺ cells but to a significantly higher extent in the ephrin-B1⁺ cells ($P < 0.05$; Wilcoxon test). Thus, the observed asymmetry is not simply due to a more general increase in tyrosine kinase activity in the EphB2⁺ cells. A third group of pTyr sites was similarly modulated in both EphB2⁺ and ephrin-B1⁺ cells; for example, phosphorylation of Y2645 on adenomatous polyposis coli (APC) was decreased to a similar extent in both cell types, whereas phosphorylation of Y15 on cyclin-dependent kinase 5 (CDK5) was similarly increased in both EphB2⁺ and ephrin-B1⁺ cells ($P > 0.85$; Wilcoxon test). Thus, signaling networks induced by contact between EphB2⁺ and ephrin-B1⁺ cells show both cell-specific and shared modes of regulation. Preferential pTyr modulation of adaptor proteins in EphB2⁺ cells suggests that these docking proteins probably have cell-specific regulatory functions. Examination of all significantly modulated pTyr sites revealed an overall increase in tyrosine phosphorylation of proteins containing Src ho-

mology 2 domain (SH2), Src homology 3 domain (SH3), or tyrosine kinase domains in EphB2⁺ cells as compared with those in ephrin-B1⁺ cells (fig. S6) (18).

Functional analysis of cell-sorting through siRNA screening. The quantitative analysis of tyrosine phosphorylation events during EphB2 and ephrin-B1 bidirectional signaling marks the activation state of signaling networks in the interacting cell populations. In order to relate these phosphoproteomic results to the phenotypic response of cell sorting and to obtain functional data for the modeling of cellular signal processing, we used an siRNA screen to analyze the role of selected signaling proteins in EphB2- and ephrin-B1-mediated cell segregation (Fig. 3). To this end, EphB2⁺ cells and ephrin-B1⁺ cells were mixed, transfected with siRNA, and grown to confluence (18). Whereas mixed control populations of EphB2⁺ and ephrin-B1⁺ cells formed distinct colonies with well-defined boundaries (fig. S7), transfection with siRNAs targeting EphB2 or ephrin-B1 significantly decreased the numbers of segregated GFP-positive colonies, indicating that cell sorting is a robust assay for EphB2-ephrin-B1 function (Fig. 3A and fig. S8).

We used a custom library of 2172 siRNA pools [SMARTpools (Dharmacon, Thermo Scientific, Lafayette, Colorado)] directed against targets with selected signaling domains, including kinases, phosphatases, and pTyr recognition modules (table S4), to screen for proteins involved in cell sorting (Fig. 3 and fig. S9B) (18). We analyzed the number of resulting EphB2⁺ GFP-positive colonies by means of automated microscopy on an Arrayscan II (Cellomics, Thermo Scientific, Pittsburgh, Pennsylvania) (fig. S9A) and identified 589 siRNAs that significantly affected

Fig. 1. Experimental outline of qBidS. (A) Cell-specific signaling in mixed populations of EphB2⁺ and ephrin-B1⁺ cells. EphB2⁺ cells were labeled with light or heavy arginine and lysine, and ephrin-B1⁺ cells were labeled with medium arginine and lysine. Bidirectional signaling was initiated, and nonstimulated light-labeled EphB2⁺ cells served as a control. (B) Mixed populations of cells were harvested after 10 min and combined with nonstimulated control cells. Cell lysates were digested with trypsin, and tyrosine-phosphorylated peptides were isolated and analyzed with LC-MS on a LTQ-Orbitrap. (C) Peptides from mixed-cell populations were differentiated and quantified via their distinct isotopic labels. In EphB2⁺ cells mixed with ephrin-B1⁺ cells, phosphorylation of the activation loop of EPHB2 at Y780 was increased by 80%, as determined from the ratio of heavy- to light-labeled peptides. Tyrosine



phosphorylation of ephrin-B1 (Y317) was only observed with a medium label, indicating that this peptide originates specifically from the ephrin-B1⁺ cells. The previously described EphB2 targets AF6 (Y1657) and SHC1 (Y427) display threefold increased levels of phosphorylation in the heavy-labeled EphB2⁺ cell population.

colony formation (the mock transfection averaged 90 colonies $\pm$ 15 within each biological replicate; $n = 4$ biological replicates) (figs. S8 and S9) (18). All affected cultures were then visually inspected, and 237 siRNAs that affect growth and adhesion were discarded. Off-target effects are inherent to siRNA technology (24), so we reanalyzed the remaining 352 targets with four individual siRNAs [ON-TARGETplus (OTP) reagents (Dharmacon, Thermo Scientific, Lafayette, Colorado)] that were distinct from those used in the initial screen and applied them to each target. Cocultures in which siRNAs recapitulated a significant effect on colony numbers were also visually confirmed (18). This approach yielded 200 targets that affect EphB2- and ephrin-B1-dependent cell sorting (fig. S8D and table S3), including several previously described effectors of EphR and ephrin signaling, such as p120RasGAP (RASA1) and the adaptor proteins CRK and NCK2. The screen also identified groups of multiple interacting proteins that potentially have a coordinated role in the regulation of aspects of EphB2-ephrin-B1 cell sorting, such as cytoskeletal organization and cell adhesion (fig. S9C). For example, activators (DOCK1 and ELMO) as well as negative regulators (CHN1 and CHN2) of RAC1 guanosine triphosphatase (GTPase) activity, in addition to RAC1 itself, were identified as targets, which suggests that tight control of RAC1 activity is important for cell sorting.

We then compared the targets identified in the siRNA screen with those containing pTyr sites modulated by the mixing of EphB2⁺ and ephrin-B1⁺ cells. Among the 200 proteins required for proper cell sorting, 37 were significantly modulated by tyrosine phosphorylation in EphB2⁺ cells and 26 in ephrin-B1⁺ cells (fig. S8E and table S3), of which 18 were significantly modulated in both cell types by phosphorylation on identical tyrosine sites (Fig. 3B and fig. S8E). These proteins identified in both the siRNA and qBidS screens have a wide range of molecular and cellular functions and include adaptor proteins (SHB and SHC1), kinases (ABL1 and EPHB2), phospholipid regulators (SHIP-2 and PLCG2), polarity proteins (PAR3, DLG3, and APC), and regulators of the cytoskeleton, endocytosis, and migration (PTK2, PXN, WASL, and ITSN2) (Fig. 3B and table S3), highlighting the role of these cellular processes in the regulation of EphR-ephrin-controlled cell sorting. The pTyr sites from the 18 targets modulated in both cell types nonetheless displayed significant differences in their cell-specific modulation ($P = 0.008$; Fisher's exact test) (fig. S9D), indicating that asymmetric tyrosine phosphorylation affects proteins that are essential for cell sorting.

Thus, EphB2- and ephrin-B1-affected changes in tyrosine phosphorylation elicit extensive changes in intracellular signaling networks. Therefore, we set out to derive a network model of cell-specific information flow on the basis of the quantified modulation of tyrosine phosphorylation and the targets identified in the siRNA screen for cell sorting.

Modeling of cell-specific dynamic networks during cell-sorting. The regulation of tyrosine phosphorylation through activated kinases and subsequent binding of pTyr sites by domains like SH2 and PTB (pTyr-binding) creates molecular circuitries (25, 26) that underlie cellular decision-making. The information flow within such

a system can therefore be visualized by assigning kinases, phosphatases, and phospho-binding modules for each pTyr site (kinase/phosphatase $\rightarrow$ substrate/site $\rightarrow$ phospho-binding domain). For example, increased phosphorylation of Tyr sites can be correlated with an increased activity of the associated kinase or kinases (or decreased activity

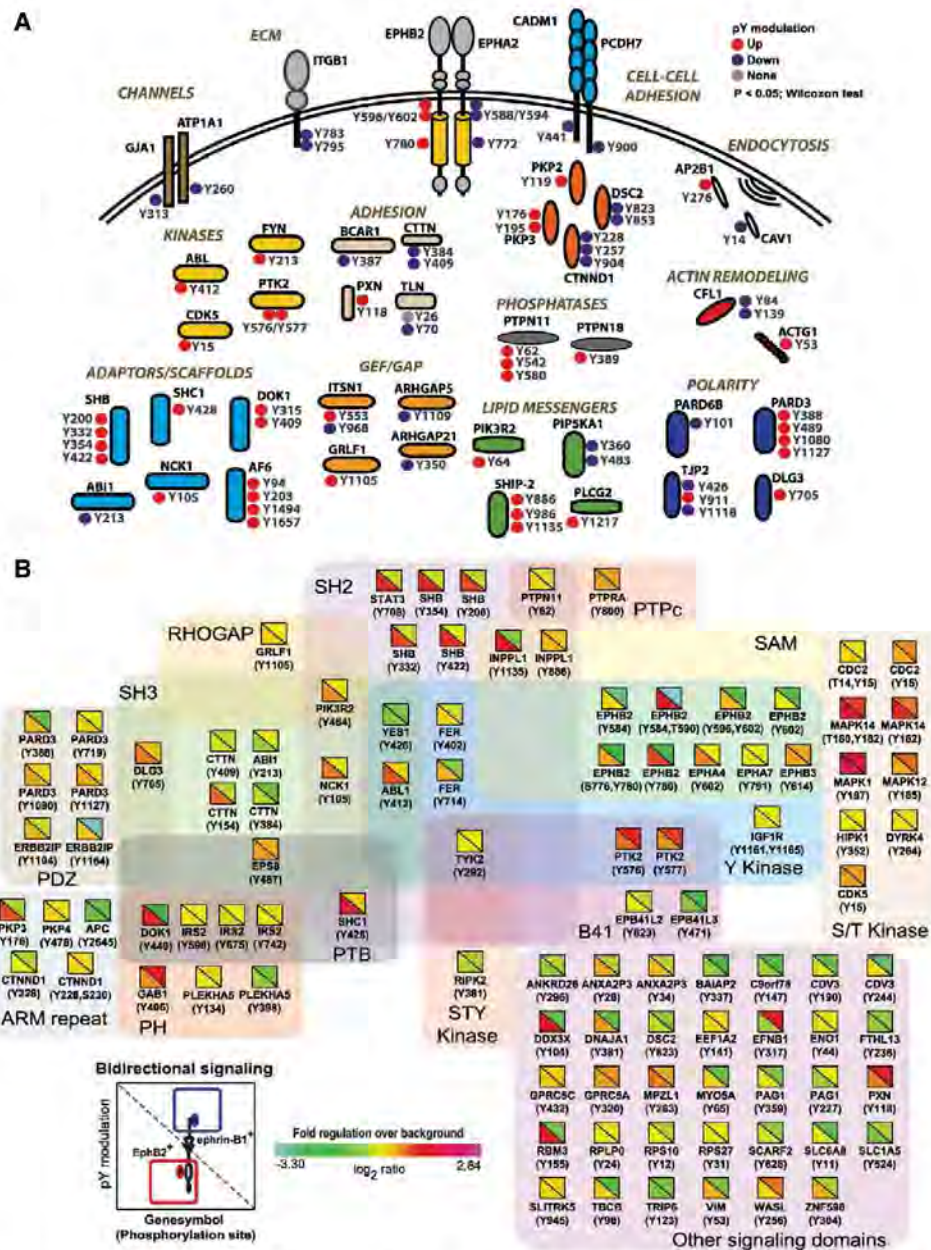


Fig. 2. Asymmetric regulation of tyrosine phosphorylation in EphB2⁺ and ephrin-B1⁺ cells. **(A)** Overview of selected proteins in EphB2⁺ cells modulated by tyrosine phosphorylation after mixing with ephrin-B1⁺ cells. The modulation of pTyr sites is indicated as significantly increased (red), not modulated (gray), or decreased (blue). Molecules that are involved in a wide variety of cellular functions, such as lipid signaling (SHIP-2, PLCG2, and PIK3R2), polarity (DLG3 and PARD3), actin and myosin turnover (NCK1 and ACTG1), endocytosis and recycling (AP2B1 and CAV1), and adaptor proteins (SHB and SHC1), as well as kinases (ABL1 and CDK5) are modulated by tyrosine phosphorylation, indicating that phospho-regulation of numerous cellular processes may be important for cell sorting. **(B)** Comparison of contact-initiated pTyr signaling between ephrin-B1⁺ and EphB2⁺ cells. Identical pTyr sites are shown grouped according to the domain composition of their host proteins. Cell-specific modulation of pTyr sites is indicated by slashed boxes, each triangle representing specific regulation within EphB2⁺ (bottom triangles) or ephrin-B1⁺ (top triangles) cells, respectively. The fold regulation of each pTyr site is shown with a color range. In total, we identified 100 identical tyrosine-phosphorylated sites in the two cell types, of which 71 displayed asymmetric modulation between EphB2⁺ and ephrin-B1⁺ cells ($P < 0.05$; Wilcoxon test).

of a phosphatase). This increase will in turn enhance the association between phosphorylated sites and proteins that contain the appropriate phospho-binding domain (27). To model the bidirectional signaling networks in EphB2⁺ and ephrin-B1⁺ cells, we used the NetworKIN and NetPhorest algorithms [http://NetworKIN.info and http://NetPhorest.info (28, 29)] to computationally reconstruct phosphorylation networks using probabilistic contextual data in combination with sequence models of kinase and phospho-binding domain consensus motifs (28, 29). We identified pTyr sites from singly phosphorylated peptides common to the two cell types that exhibited

significant modulation after cell-cell contact ($P < 0.05$; Wilcoxon test). Accurate modeling of phosphorylation networks requires contextual information for both kinases and substrates (28). Therefore, we developed four filtering steps to restrict the predicted kinases and phospho-binding proteins to those more likely to be relevant for cocultured EphB2–ephrin-B1–expressing HEK293 cells and to limit spurious predictions: First, predicted kinases or phospho-binding proteins were accepted only if they had been previously identified by the qBidS or siRNA screens (Fig. 4). Second, we required that kinases and substrates, which were predicted to interact,

were similarly modulated (up, down, or none) (Fig. 4, co-modulation). The activities of kinases were determined by the modulation of their activation loop phosphorylation sites and correlated to the phosphorylation of substrate sites. Third, predictions were ranked and filtered on the basis of their probability from NetPhorest (Fig. 4) (18, 29). We also analyzed the predicted kinase-substrate-target relationships by overlaying a HEK293-specific contextual network (Fig. 4) (18). The latter was generated with proteins identified through qBidS, siRNA screening, or coimmunoprecipitation with selected network proteins (table S5) (18) as seed input data to the Search

Fig. 3. Functional siRNA screening of EphB2- and ephrin-B1-regulated cell sorting. (A) EphB2⁺ cells, which coexpress myristoylated GFP, were mixed with ephrin-B1⁺ cells, transfected with siRNA pools, and grown to 100% density. The number of GFP-positive EphB2⁺ colonies was used to determine the effect of siRNAs on cell sorting. Disruption of EphB2 or ephrin-B1 expression by means of siRNA inhibits colony formation. (B) Proteins identified through siRNA screening as functionally important for cell sorting also tend to be asymmetrically phosphorylated. The modulation of pTyr sites residing in proteins important for cell sorting that were identified in both EphB2⁺ and ephrin-B1⁺ cells was compared. The number of OTP duplexes recapitulating a loss of cell sorting is shown to the left in red, followed by the gene name, the identified pTyr sites, and the cellular function of the protein. The cell-specific modulation of each pTyr site is shown by color code.

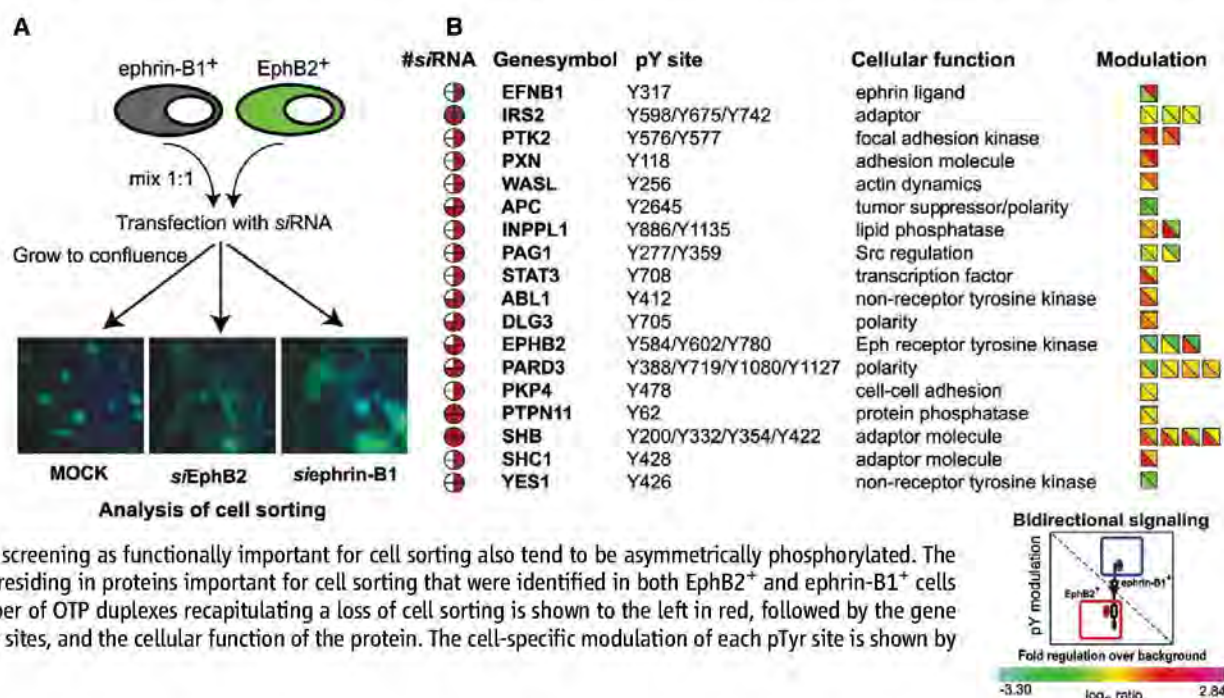
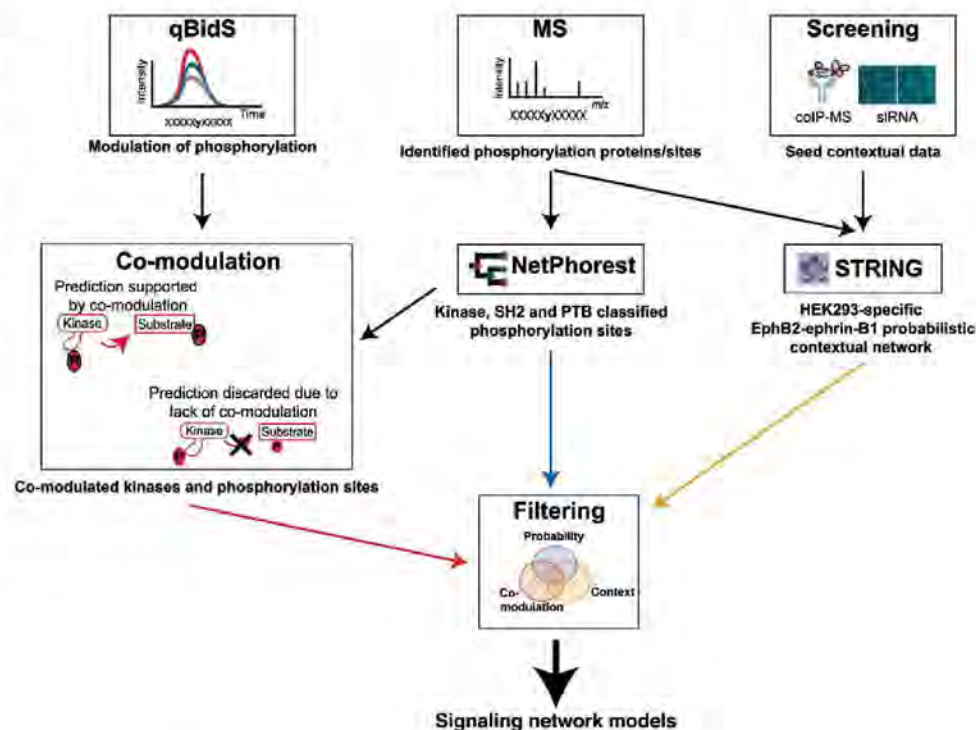


Fig. 4. Computational data integration and network modeling. Systems-specific data integration was performed to construct network models. All observed phosphorylation sites were first processed by the NetPhorest algorithm (29) so as to predict kinase-substrate relationships and SH2 and PTB domain interactions. These predictions were subjected to several subsequent filtering schemes: First, we required that tyrosine phosphorylation sites and the activation loop phosphorylation of their predicted kinases be comodulated (red path). Second, predictions were filtered on the basis of the probability score from Netphorest (blue path). Proteins that were identified through qBidS or siRNA screening or by coprecipitation were used as input to the STRING resource to generate a systems-specific protein-protein interaction network, permitting contextual filtering (orange path) similar to the NetworkKIN algorithm (28). This enabled the generation of cell-specific signaling network models.



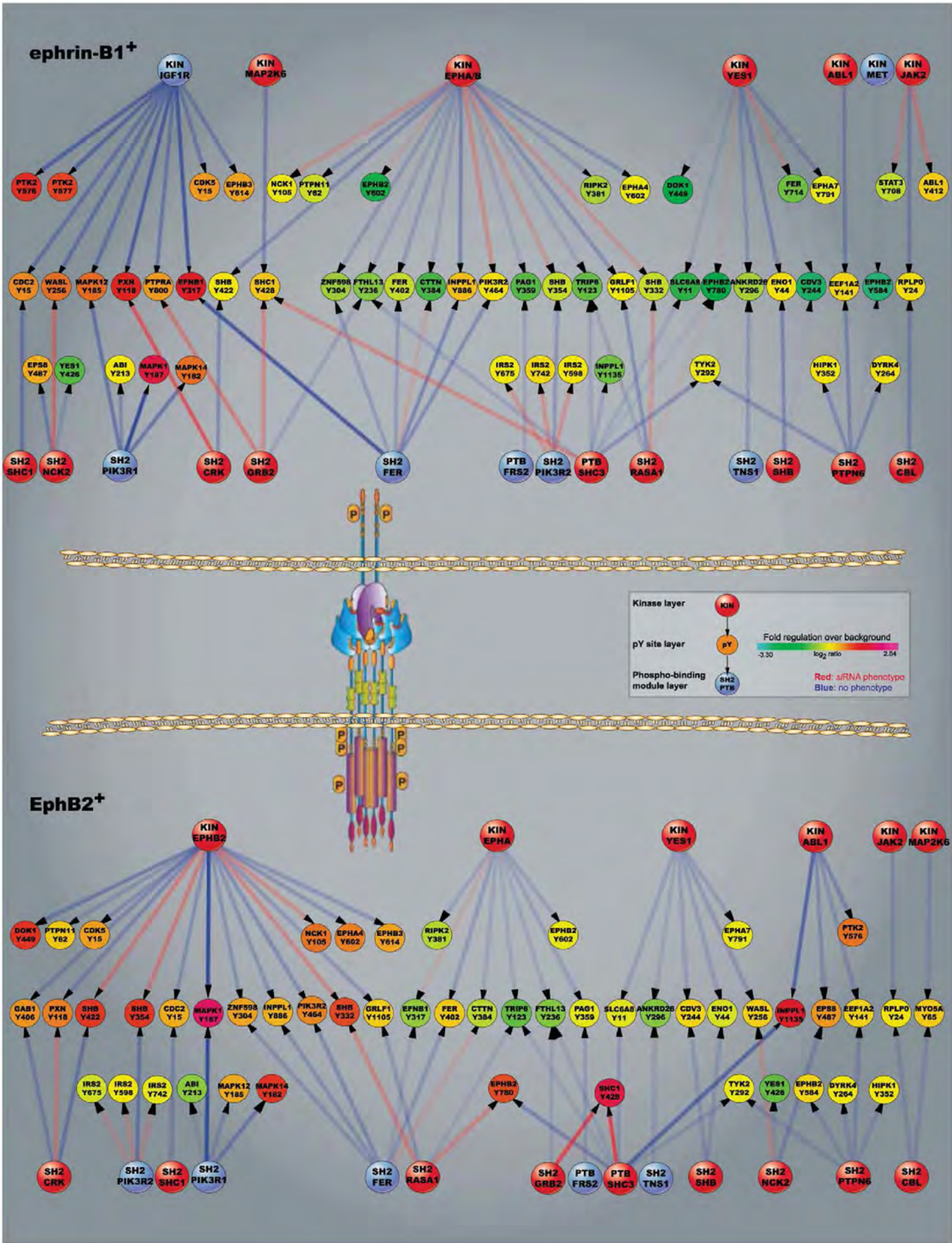


Fig. 5. Cell-specific signaling network models in EphB2- and ephrin-B1-expressing cells. Cell-specific information flow in EphB2⁺ and ephrin-B1⁺ cells is shown in the form of modular protein networks with kinases (KIN), pTyr sites, and phospho-binding modules (SH2 and PTB) organized in layers. The predicted kinases and phospho-binding modules for each tyrosine-phosphorylated residue are shown. The color of the pTyr sites represents their cell-specific modulation, according to the indicated color code, whereas the color of each kinase and phospho-binding node represents whether through siRNA it was identified as a cell-sorting

target (red) or not (blue). The arrows represent the strength of the information flow within the network and the intensity is proportional to the modulation of the pTyr site involved in the specific edge. Arrows color-coded red represent an interaction supported by the contextual protein association network. Different kinases between the EphB2⁺ and ephrin-B1⁺ cells appear to be responsible for the change in information flow, and it appears that SH2- and PTB-binding proteins are used to a larger degree in EphB2⁺ cells than in ephrin-B1⁺ cells. Only a subset of the network is shown; for the full network, see the SOM.

Tool for the Retrieval of Interacting Genes/Proteins (STRING) resource (30) so as to create a probabilistic high-confidence protein-protein interaction network. Thus, by applying stringent filters we generated a cell-specific model of information processing during bidirectional signaling between mixed EphB2⁺ and ephrin-B1⁺ cells (Fig. 5).

Cell-specific signal processing in Eph receptor and ephrin signaling networks. To reflect the flow of information through pTyr sites, we assembled cell-specific networks in a modular fashion so that kinases (top layer) were connected through pTyr sites (middle layer) to phospho-binding modules (bottom layer) (Fig. 5). The signal strength through the connected kinases, pTyr sites, and phospho-binding modules is reflected by the modulation of the individual pTyr sites and represented by the intensity of the line (indicating an interaction) (Fig. 5).

Previously characterized pTyr-dependent protein-protein interactions involved in regulating cytoskeletal rearrangement and cell migration were confirmed by the model (Fig. 5). For example, phosphorylated EPHB2 displays increased interaction with RASA1 and DOK1 after EphR activation (31). Phosphorylation of Y428 on SHC1 creates a well-described binding site for the adaptor protein GRB2 after activation of receptor tyrosine kinases (RTKs) (32). Association between cortactin (CTTN, Y384) and the tyrosine kinase FER is important for subsequent phosphorylation and regulation of CTTN function in cell-cell signaling (33). As well, an interaction between tyrosine-phosphorylated p190RhoGAP (GRFL1) and RASA1 regulates cellular migration (34). Previously undescribed pTyr-dependent interactions are also suggested by the model, such as interactions between tyrosine-phosphorylated EPHB2 and the SH2 domain of the adaptor protein SHB and the tyrosine-phosphorylated SHB with the SH2 domain of RASA1.

A global comparison of cell-specific signaling networks suggests that asymmetric regulation of tyrosine phosphorylation events between EphB2⁺ and ephrin-B1⁺ cells is achieved through alternative use of kinases and adaptor proteins, thereby providing alternative signaling paths to relay information. Most of the pTyr signaling in EphB2⁺ cells originates from the EPHB2 and ABL1 kinases, with NCK1, PXN, DOK1, and SHB as predicted direct targets (Fig. 5). Conversely, in ephrin-B1⁺ cells, the insulin-like growth factor receptor (IGF1R) is predicted to initiate some of the pTyr signaling, which is in agreement with the observation that both the activation loop

(Y1161) of IGF1R and its target IRS2 display increased tyrosine phosphorylation in ephrin-B1⁺ cells after stimulation. Currently, we cannot exclude the possibility that other tyrosine kinases contribute to the observed pTyr regulation. Although other possible kinases were predicted for the positively modulated pTyr sites, we only obtained experimental evidence of increased activation loop phosphorylation for IGF1R and PTK2 in ephrin-B1⁺ cells.

Consistent with SH2 and PTB domain-containing proteins displaying differential phosphorylation in EphB2⁺ and ephrin-B1⁺ cells (Fig. 2B), the network model shows asymmetric utilization of adaptor proteins. For example, several of the direct targets predicted for EPHB2 and ABL1 contain a pTyr binding module (SH2 or PTB), whereas less information is processed through the phosphorylation of SH2 and PTB domain-containing proteins in ephrin-B1⁺ cells. SHB, an adaptor protein that has not previously been characterized with respect to EphR-ephrin signaling, appears to relay information from EPHB2 to CRK, RASA1, and PIK3R1. The asymmetric modulation of SHB tyrosine phosphorylation, coupled with the inhibitory effects of SHB siRNA on cell sorting, support the prediction that SHB may act as a crucial signal integrator (Figs. 3B and 5).

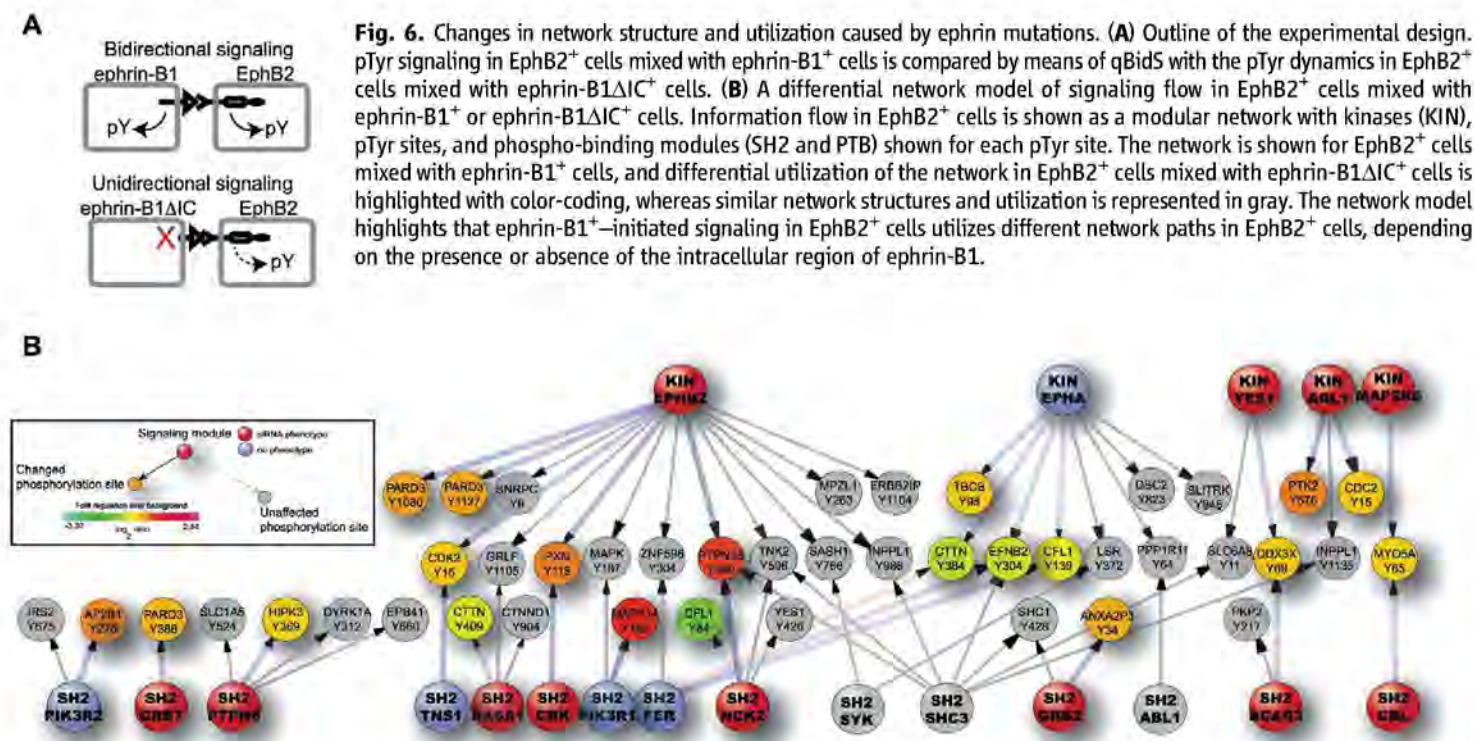
The global comparison of pTyr modulation in ephrin-B1⁺ and EphB2⁺ cells showed that a similar number of pTyr sites display negative or positive modulation (fig. S5A). Consistent with this observation, the network model identified several tyrosine phosphatases that may have a role in balancing pTyr signaling. Although tyrosine phosphatases have been described in EphR-ephrin signaling (17, 35–37), the model implicates protein tyrosine phosphatase (PTP) non-receptor type 6 (PTPN6) and PTP receptor type A (PTPRA) as previously undescribed regulators of this process in addition to PTPN11, which has been linked to EphA receptor signaling. PTPN6 and PTPN11, which according to the network model are in close proximity to EPHB2, were identified in the siRNA screen, and PTPN11 phosphorylation (Y62) was modulated in an EphB2⁺ cell-specific manner, suggesting that these phosphatases probably function in EphB2 and ephrin-B1 signaling and cell sorting.

Lastly, when comparing the signaling network models with the siRNA screen, we observed a strong bias toward highly connected signaling proteins exhibiting an siRNA phenotype ($P = 0.001$ for EphB2⁺ cells and $P = 0.002$ for ephrin-B1⁺ cells; Wilcoxon test) (fig. S10) (18).

Signaling abilities of Eph and ephrin intracellular region. Ephrin-B1, lacking its cytoplasmic region, retains the ability to initiate signaling through a B-type EphR on a neighboring cell but lacks intrinsic signaling properties and thus only elicits a unidirectional signal (Fig. 6A) (4). The size of the signaling cluster established between interacting EphRs and ephrins can markedly affect receptor activation and signaling (36), and so the intracellular region of ephrin-B1 might affect the strength or quality of the signal within the EphB2-expressing cell through its ability to associate with SH2 or PDZ domain-containing proteins.

To systematically study this, we engineered HEK293 cells to express a variant of ephrin-B1 lacking the cytoplasmic tail (ephrin-B1ΔIC⁺) and selected cells with a similar level of surface expression as that observed in ephrin-B1⁺ cells (fig. S1) (18). We then mixed EphB2⁺ cells with either ephrin-B1ΔIC⁺ cells or ephrin-B1⁺ cells (expressing wild-type ephrin-B1) and used qBidS to compare their sorting phenotypes and the resulting pTyr signals. This revealed that the intracellular tail of ephrin-B1 is required for proper cell sorting and also influences pTyr signaling in EphB2⁺ cells (Fig. 6B and figs. S7 and S11) (18). Specifically, pTyr sites in EphB2⁺ cells were differentially modulated depending on whether the EphB2⁺ cells were stimulated with ephrin-B1ΔIC⁺ or ephrin-B1⁺ cells ($P = 9 \times 10^{-19}$; Wilcoxon test) (fig. S12B), indicating that the intracellular region of ephrin-B1 affects EphB2 signaling. For example, cofilin 1 [CFL-1 (Y139 and Y84)], EPHB2 (Y596/Y602), PARD3 (Y388 and Y1127), PXN (Y118), and PTK2 (Y576) were differentially phosphorylated in EphB2⁺ cells depending on the presence or absence of the cytoplasmic region of ephrin-B1 ($P < 0.05$; Wilcoxon test) (fig. S11). The qBidS approach can therefore identify and quantitate differences in signaling events in a target cell that result from alterations in a distinct signal-initiating cell (thus, a non-cell-autonomous effect).

We also observed major differences in the structure and utilization of the signaling network in EphB2⁺ cells depending on whether they were mixed with ephrin-B1⁺ or ephrin-B1ΔIC⁺ cells (Fig. 6B). Nodes and edges in the EphB2⁺ cell signaling network that are influenced by the cytoplasmic tail of ephrin-B1 are depicted in a color-coded scheme, and those that were utilized similarly are in gray. The information flow from all predicted kinases and nearly all phospho-binding domains was affected when EphB2⁺ cells were mixed with ephrin-B1ΔIC⁺ cells as



opposed to ephrin-B1⁺ cells. For example, whereas the predicted kinase for some pTyr sites such as PARD3 (Y1127) remained unaltered, other pTyr sites such as CTTN (Y384), PARD3 (Y1080), and PXN (Y118) were predicted to be modulated by an alternative kinase, suggesting that the dynamics and structure of the EphB2 signaling network is significantly influenced by the intracellular region of ephrin-B1 (Fig. 6D) [detailed networks are available in the supporting online material (SOM)].

Taken together, these data show that the intracellular region of ephrin-B1 affects signal processing not only within ephrin-B1⁺ cells but also in neighboring cells that express EphB2, revealing a non-cell-autonomous mode of regulation in EphR-ephrin signaling. The same applies to the C-terminal valine of ephrin-B1 required for PDZ domain-binding of ephrin-B1, which also influenced signaling in EphB2⁺ cells (fig. S13) (18). Furthermore, pTyr signaling is differently modulated in EphB2⁺ cells depending on whether they are mixed with ephrin-B1ΔIC⁺ or ephrin-B1ΔV⁺ cells (Fig. 6 and figs. S11 and S13), suggesting that additional components besides PDZ domain-containing proteins may be required to orchestrate the signaling effects of ephrin-B1 on EphB2. The cytoplasmic region of EphB2 influences signaling in ephrin-B1⁺ cells (fig. S14). We observed a significantly different pTyr-signaling response when EphB2⁺ or ephrin-B1⁺ cells were stimulated by soluble fusion proteins containing the extracellular regions of ephrin-B1 or EphB2, respectively, as compared with coculture (figs. S5, S15, and S16) (18).

Discussion. We developed proteomic and computational approaches to identify cell-specific signaling networks in two cell populations as they

contact one another and explored the phenotypic impact of these networks on cell sorting. Our models show that cell segregation requires the integration of multiple cellular processes. We found that information processing between EphB2⁺ and ephrin-B1⁺ cells is asymmetric and that there are both structural and dynamic differences in the networks mediating the molecular information flow induced by bidirectional signaling on the one hand, as compared with unidirectional signaling induced either by C-terminally truncated cell surface ligands, or soluble proteins on the other. One aim of integrative network biology is to model biological systems with an accuracy and predictive power similar to that of other physical systems, and thus it is essential to intertwine quantitative measurements of cell behavior and signaling dynamics through computational modeling (16, 38, 39–41). The availability of more diverse and larger quantitative phenotypic, proteomic, and interaction data, with improved algorithms (26, 27, 38), will enable more accurate and elaborate signaling models to be generated. The systems-level approaches described here are of general utility in studying the effects of cell-cell interactions and network utilization in both normal and pathologic processes.

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Human Frontiers Science Program, and The Lundbeck Foundation. C.J. conceived and implemented the qBids approach. R.L. conceived and performed the modeling work. C.J., A.S., and T.P. conceived and designed the siRNA screen. D.G.W. and A.P. (MRC NIMR) provided cell lines and advice on cell sorting. C.J., A.S., G.I.C., M.H., and B.L. conceived, designed, and performed the experiments. A.P., C.J., and R.L. conceived, designed, and performed the computational experiments. T.P. and R.L. oversaw the project. C.J., R.L., and T.P. wrote the paper. Mass spectrometric data has been submitted to the Proteomics Identifications (PRIDE) database under accession numbers 10295–10296.

Identified phosphorylation sites have also been submitted to the Phospho-ELM and PhosphoSite databases.

Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S21

Tables S1 to S5

References

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Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors

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The pathogenicity of many bacteria depends on the injection of effector proteins via type III secretion into eukaryotic cells in order to manipulate cellular processes. TAL (transcription activator–like) effectors from plant pathogenic *Xanthomonas* are important virulence factors that act as transcriptional activators in the plant cell nucleus, where they directly bind to DNA via a central domain of tandem repeats. Here, we show how target DNA specificity of TAL effectors is encoded. Two hypervariable amino acid residues in each repeat recognize one base pair in the target DNA. Recognition sequences of TAL effectors were predicted and experimentally confirmed. The modular protein architecture enabled the construction of artificial effectors with new specificities. Our study describes the functionality of a distinct type of DNA binding domain and allows the design of DNA binding domains for biotechnology.

Phytopathogenic bacteria of the genus *Xanthomonas* cause severe diseases on many crop plants. Pathogenicity relies on the translocation of effector proteins into the plant cell cytoplasm via the type III secretion system (1–5). Members of the large transcription activator–like (TAL) effector family are key virulence factors of *Xanthomonas* (4–7) and reprogram host cells by mimicking eukaryotic transcription factors (8–13). TAL effector–mediated gene induction leads to plant developmental changes [for example, cell divisions and cell enlargement such as citrus canker and hypertrophy (4)], thus contributing to disease symptoms. Although a number of plant targets, including susceptibility genes, have been identified (8–10, 12–14), the targets of most TAL effectors have not been elucidated.

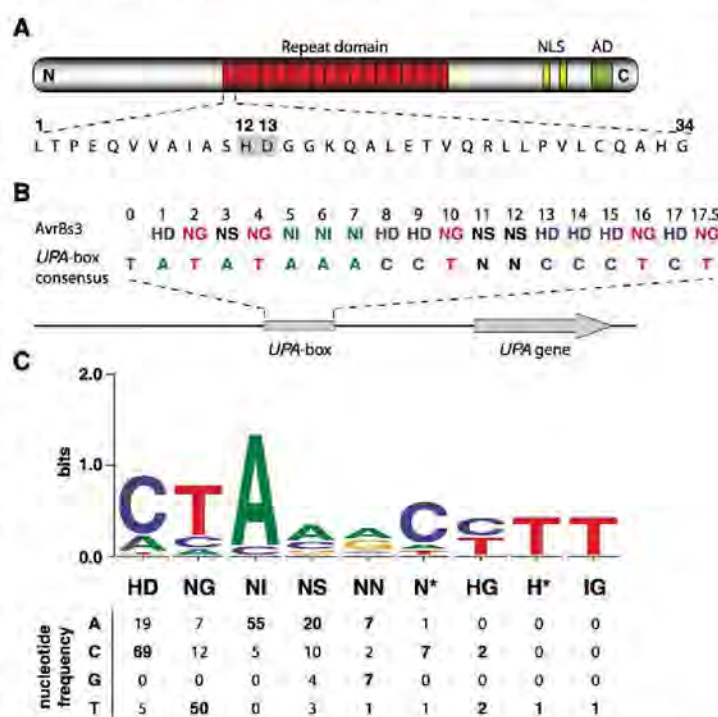
TAL effectors are characterized by a central domain of tandem repeats, nuclear localization signals (NLSs), and an acidic transcriptional activation domain (AD) (Fig. 1A) (11, 15, 16). Members of this effector family are highly conserved and differ mainly in the amino acid sequence and number of repeats. The number and order of re-

peats in a TAL effector determine its specific activity (17, 18). The type member of this effector family, AvrBs3 from *Xanthomonas campestris* pv.

vesicatoria, contains 17.5 repeats and induces expression of *UPA* (upregulated by AvrBs3) genes, including the *Bs3* resistance gene in pepper plants (9, 10, 14, 19). The repeats of AvrBs3 are essential for DNA binding of AvrBs3 and represent a distinct type of DNA binding domain (9). How this domain contacts DNA and what determines specificity has remained enigmatic so far.

A model for sequence specificity. The fact that AvrBs3 directly binds to the *UPA* box, a promoter element in induced target genes (9, 10), prompted us to investigate the basis for DNA–sequence specificity. The repeat region of AvrBs3 consists of 34 amino acid repeat units that are nearly identical; however, amino acids 12 and 13 are hypervariable (Fig. 1A) (11). The most C-terminal repeat of AvrBs3 shows a sequence similarity to other repeats only in its first 20 amino acids and is therefore referred to as a half repeat. The repeats can be classified into different repeat types on the basis of their hypervariable 12th and 13th amino acids (Fig. 1B). Because the size of the *UPA* box [18 base pairs (bp)] (20)

Fig. 1. Model for DNA–target specificity of TAL effectors. (A) TAL effectors contain central tandem repeats, NLSs, and an AD. Shown is the amino acid sequence of the first repeat of AvrBs3. Hypervariable amino acids 12 and 13 are shaded in gray. (B) Hypervariable amino acids at position 12 and 13 of the 17.5 AvrBs3 repeats are aligned to the *UPA* box consensus (14). (C) Repeats of TAL effectors and predicted target sequences in promoters of induced genes were aligned manually. Nucleotides in the upper DNA strand that correspond to the hypervariable amino acids in each repeat were counted on the basis of the following combinations of eight effectors and experimentally identified target genes: AvrBs3/*Bs3*, *UPA10*, *UPA12*, *UPA14*, *UPA19*, *UPA20*, *UPA21*, *UPA23*, *UPA25*, AvrBs3Δrep16/*Bs3*-E, AvrBs3Δrep109/*Bs3*, AvrHah1/*Bs3*, AvrXa27/*Xa27*, PthXo1/*Xa13*, PthXo6/*OstFX1*, and PthXo7/*OstFIIAγ1* (fig. S1). An asterisk indicates that amino acid 13 is missing in this repeat type. Highest nucleotide frequencies are in bold. Nucleotide frequencies are displayed in a logo (<http://weblogo.threeplusone.com>).



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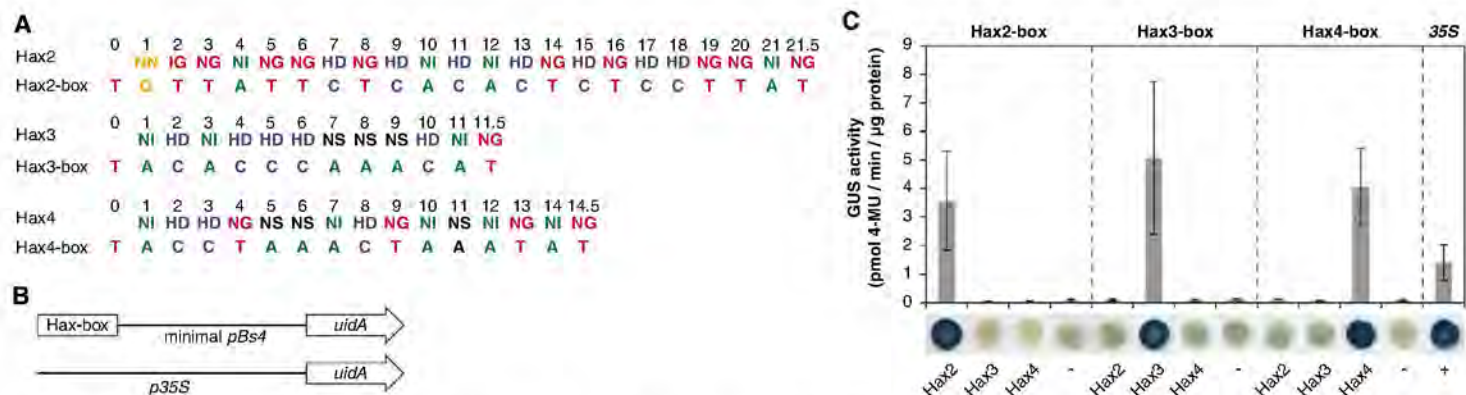
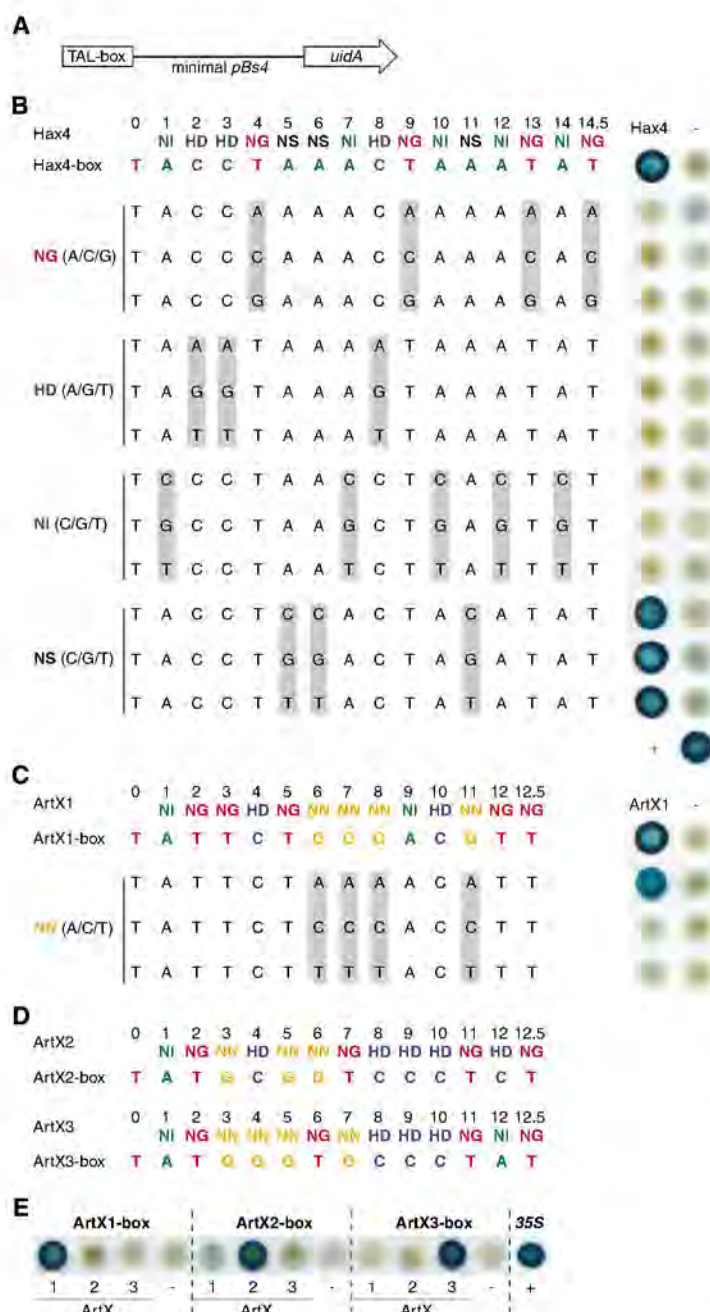


Fig. 2. Target DNA sequences of Hax2, Hax3, and Hax4. **(A)** Amino acids 12 and 13 of the Hax2, Hax3, and Hax4 repeats and predicted target DNA specificities (Hax box). **(B)** Hax boxes were cloned in front of the minimal *Bs4* promoter into a GUS reporter vector. **(C)** Specific inducibility of the Hax boxes by Hax effectors. GUS

reporter constructs were codelivered via *A. tumefaciens* into *N. benthamiana* with 35S-driven *hax2*, *hax3*, *hax4*, and empty T-DNA (–), respectively (error bars indicate SD; $n = 3$ samples). 4-MU, 4-methyl-umbelliferone. 35S::uidA (+) served as control. Leaf discs were stained with X-Gluc (5-bromo-4-chloro-3-indolyl- β -D-glucuronide).

Fig. 3. DNA base pair recognition specificities of repeat types. **(A)** Hax4 and ArtX box derivatives were cloned in front of the minimal *Bs4* promoter into a GUS reporter vector. **(B)** Specificity of NG, HD, NI, and NS repeats. Hax4-inducibility of Hax4 box derivatives permutated in repeat type target bases (gray background). **(C)** Specificity of NN repeats. Artificial effector ArtX1 and predicted target DNA sequences. ArtX1-inducibility of ArtX1 box derivatives permutated in NN repeat target bases (gray background). **(D)** Artificial effectors ArtX2 and ArtX3 and derived DNA target sequences. **(E)** Specific inducibility of ArtX boxes by artificial effectors. **(A)** to **(E)** GUS reporter constructs were codelivered via *A. tumefaciens* into *N. benthamiana* with 35S-driven *hax4*, *artX1*, *artX2*, or *artX3* genes, and empty T-DNA (–), respectively. 35S::uidA (+) served as control. Leaf discs were stained with X-Gluc. For quantitative data, see fig. S8.



or 19 bp (14) almost corresponds to the number of repeats (17.5) in AvrBs3, we considered the possibility that one repeat unit of AvrBs3 contacts one specific DNA base pair. When the repeat types of AvrBs3 (amino acid 12 and 13 of each repeat) are projected onto the *UPA* box, it becomes evident that certain repeat types correlate with specific base pairs in the target DNA. For example, HD and NI repeats have a strong preference for C and A, respectively (Fig. 1B) (21). For simplicity, we designate here only bases in the upper (sense) DNA strand. Our model of recognition specificity is supported by the fact that the AvrBs3 repeat deletion derivative AvrBs3 Δ rep16, which lacks four repeats (11 to 14) (fig. S1, A and B) (17), recognizes a shorter and (in the 3' part of the box) different target DNA sequence (figs. S1 to S4) (10, 14, 20). On the basis of sequence comparisons of *UPA* boxes of AvrBs3-induced pepper genes (14) and mutational analysis (20), the target DNA box of AvrBs3 appeared to be 1 bp longer than the number of repeats in AvrBs3. In addition, a T is conserved at the 5' end of the *UPA* box immediately preceding the predicted recognition specificity of the first repeat (Fig. 1). Secondary structure predictions of the AvrBs3 amino acid sequence preceding the first repeat and the repeat region (11) show similarities, despite the lack of sequence conservation. This suggests an additional repeat, here termed repeat 0, that extends the target boxes of AvrBs3 and possibly other TAL effectors at their 5' ends (Fig. 1B).

Challenging the code. To further substantiate and extend our model (Fig. 1B), we predicted the yet unknown target DNA sequences of *Xanthomonas* TAL effectors (AvrXa27, PthXo1, PthXo6, and PthXo7 from *Xanthomonas oryzae* pv. *oryzae*) on the basis of the sequence of their repeats and inspected the promoters of known target genes and their alleles for the presence of putative binding sites. We identified sequences matching the predicted specificity in promoters of alleles that are induced in response to the corresponding TAL effector but not in noninduced alleles (fig. S1, C to

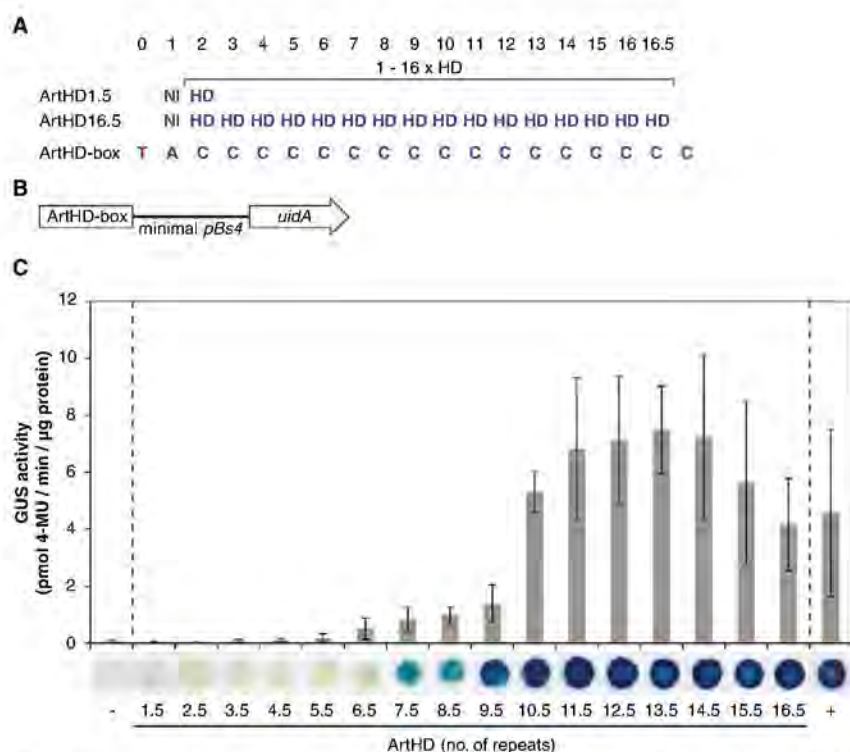


Fig. 4. A minimal number of repeats is required for transcriptional activation. (A) Artificial ArtHD effectors with different numbers (0.5 to 15.5) of HD repeats (total of 1.5 to 16.5 repeats). (B) An ArtHD target box consisting of TA and 17 C was cloned in front of the minimal *Bs4* promoter into a GUS reporter vector. (C) Promoter activation by ArtHD effectors with different number of repeats. 35S-driven effector gene or empty T-DNA (–) were codelivered via *A. tumefaciens* with the GUS-reporter construct into *N. benthamiana* (error bars indicate SD; $n = 3$ samples 4-MU). 35S::uidA (+) served as control. Leaf discs were stained with X-Gluc.

F). The presence of these target boxes suggests that the induced genes are direct targets of the corresponding TAL effectors. On the basis of the DNA base frequency for different repeat types in the target DNA sequences by using eight TAL effectors (fig. S1), we predicted a code for the DNA target specificity of their repeat types (Fig. 1C).

To experimentally validate our model, we predicted target DNA sequences for the TAL effectors Hax2 (21.5 repeats), Hax3 (11.5 repeats), and Hax4 (14.5 repeats) from the *Brassicaceae*-pathogen *X. campestris* pv. *armoraciae* (22, 23). Hax2 is exceptional, because it has 35-amino acid instead of the typical 34-amino acid repeats. The Hax2, Hax3, and Hax4 target boxes were placed in front of the minimal (–55 to +25) tomato *Bs4* promoter, which has very weak basal activity (Fig. 2B and figs. S5 and S6) (24), driving a promoterless *uidA* [β -glucuronidase (*GUS*)] reporter gene. For transient expression studies, we transfected the reporter constructs together with the constitutive cauliflower mosaic virus 35S-promoter-driven effector genes *hax2*, *hax3*, and *hax4* into *Nicotiana benthamiana* leaves using *Agrobacterium*-mediated transfer DNA (T-DNA) delivery. Qualitative and quantitative GUS assays demonstrated that promoters containing the predicted Hax2, Hax3, or Hax4 box were only induced in the presence of the corresponding effector (Fig. 2C and fig. S6). This validates our model for DNA recognition by TAL effectors and the code for DNA target specificity of their repeat

types. In addition, these data demonstrate that 35-amino acid repeats function like 34-amino acid repeats.

Nucleotide specificity of repeat types. Next, we addressed the importance of the first nucleotide (T; corresponding to repeat 0) in the predicted target DNA sequence for Hax3 and generated four different Hax3 boxes with either A, C, G, or T at the 5' end (fig. S7, A and B). Codelivery of *hax3* and the reporter constructs in *N. benthamiana* demonstrated that only a promoter containing a Hax3 box with a 5' T was induced in the presence of Hax3 (fig. S7C). This indicates that a T at position 0 contributes to promoter activation of Hax3 and probably other TAL effectors.

To address the question of whether the repeat types are specific and recognize only one specific base pair, respectively, we permuted the Hax4 box (Fig. 3, A and B). GUS assays showed that NI, HD, and NG repeats in Hax4 strongly favor recognition of A, C, and T, respectively, whereas NS repeats recognize all four base pairs (Fig. 3B and fig. S8).

Because several TAL effectors contain NN repeats (fig. S1 and table S1) (11) for which recognition specificity has not been tested, we generated ArtX1, an artificial TAL effector with 12.5 randomly assembled repeats that include NN repeats and deduced a corresponding DNA recognition sequence using our code (Figs. 1C and 3C). Analysis of ArtX1 box derivatives demonstrated that NN repeats recognize both A and G,

with preference for G (Fig. 3C and fig. S8). This result confirms our prediction of the Hax2 box (Fig. 2) and the natural *AvrXa27* box in the promoter of the rice *Xa27* gene, which contains either an A or a G at positions corresponding to NN repeats (fig. S1C). In addition, we derived two possible *AvrXa10* boxes with either A or G at positions corresponding to NN repeats in *AvrXa10*. Both reporter constructs were induced efficiently by *AvrXa10* (fig. S9). Together, these data suggest that some repeat types favor recognition of specific base pairs, whereas others are more flexible.

Prediction of target genes. The expression of *hax2* in *Arabidopsis thaliana* leads to purple colored leaves, indicating an accumulation of anthocyanin (fig. S10, A and B). To identify Hax2 target genes, we analyzed promoter regions of the *A. thaliana* genome using pattern search [Patternmatch, The Arabidopsis Information Resource (TAIR); www.arabidopsis.org] with degenerated Hax2 box sequences. One of the putative Hax2 target genes encodes the MYB transcription factor PAP1 (At1G56650), which controls anthocyanin biosynthesis (25). Pattern search revealed a suboptimal Hax2 box in the *PAP1* promoter region (fig. S10, D and E). Semiquantitative analysis of the *PAP1* transcript level demonstrated that expression of *PAP1* is strongly induced by Hax2 (fig. S10C). This demonstrates that we can successfully predict target genes for TAL effectors. On the basis of the code for repeat types (Fig. 1D) and the data described above, we predicted putative target DNA sequences for additional TAL effectors, some of which are important virulence factors (table S1).

The number of repeats. Because the repeat number in TAL effectors ranges from 1.5 to 28.5 (4), a key question is whether TAL effectors with few repeats can activate gene expression. Therefore, we tested how the number of repeats influences target gene expression. For this, we constructed N-terminal green fluorescent protein (GFP) fusions of artificial effectors that contain the N- and C-terminal regions of Hax3 and a repeat domain with 0.5 to 15.5 HD repeats (specificity for C). The HD repeats were cloned into an *Esp31* site located after the codons for amino acid 12 and 13 of the first Hax3 repeat (NI repeat). Therefore, the first repeat in all cases was NI (specificity for A). The corresponding target DNA box consists of 17 C-residues preceded by TA (Fig. 4, A and B). Promoter activation by the artificial effectors was measured by use of the transient *Bs4*-promoter GUS assay in *N. benthamiana*. Although at least 6.5 repeats were needed for gene induction, 10.5 or more repeats led to strong reporter gene activation (Fig. 4C). Confocal microscopy revealed that all effectors localized to the plant cell nucleus, indicating production of full-length proteins. These data demonstrate that a minimal number of repeats is required to recognize the target DNA box and efficiently activate gene expression. The results also suggest that TAL effectors with fewer repeats are largely inactive.

Artificial effectors with implications for biotechnology. We have shown that the repeat region of TAL effectors has a sequential nature that corresponds to a consecutive target DNA sequence. Hence, it should be feasible to generate effectors with novel DNA binding specificities. Seven artificial effectors (ArtX) were generated, three with 10.5 and four with 12.5 randomly assembled repeats. They were constructed as N-terminal fusions to GFP and tested for induction of *Bs4* promoter-reporter fusions containing predicted target DNA sequences. All seven effectors induced the GUS reporter only in the presence of the corresponding target DNA box (three are shown in Fig. 3, C to E, and fig. S8). The effectors showed GFP fluorescence exclusively in the plant cell nucleus after *Agrobacterium*-mediated expression, indicating the production of full-length proteins. This shows that we are able to design DNA binding domains that target a specific DNA sequence.

Our code for recognition specificity of TAL effectors solved a 20-year enigma dating from the cloning of *avrBs3*, the first TAL effector gene (26). The repeat region mediates direct binding to DNA (9). Here, we discover how target specificity is encoded. One repeat corresponds to one base pair in the DNA, and the tandem array of repeats corresponds to a consecutive DNA sequence. Target DNA specificity is based on a two-amino acid motif per repeat, enabling the deduction of a simple code to predict the DNA target preference of TAL effectors. We have experimentally identified the following recognition preferences: HD = C; NG = T; NI = A; NS = A, C, G, or T; NN = A or G; and IG = T. Because many TAL effectors are ma-

ior virulence factors (4, 5), the knowledge of host targets will enhance our understanding of plant disease development caused by xanthomonads.

In addition, we successfully designed artificial TAL effectors that act as transcription factors with novel DNA-binding specificities. Zinc finger transcription factors, which encode DNA binding specificity in their tandem zinc finger units, have been engineered to bind chosen DNA sequences, leading to gene control systems of great utility for biotechnology (27, 28). Similarly, the TAL effectors use a DNA binding code that can be exploited to generate DNA binding domains for any DNA target.

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21. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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29. We thank J. Streubel, A. Richter, and P. Römer for kindly providing constructs; T. Gonzalez for advice on anthocyanin synthesis; and R. Kahmann for helpful suggestions on the manuscript. A patent covering the findings is pending. This work was supported by grants from the Deutsche Forschungsgemeinschaft (SPP 1212 to J.B. and U.B. and SFB 648 to U.B.).

Supporting Online Material

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Materials and Methods

Figs. S1 to S10

Table S1

References

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REPORTS

Modulated High-Energy Gamma-Ray Emission from the Microquasar Cygnus X-3

The Fermi LAT Collaboration*†

Microquasars are accreting black holes or neutron stars in binary systems with associated relativistic jets. Despite their frequent outburst activity, they have never been unambiguously detected emitting high-energy gamma rays. The Fermi Large Area Telescope (LAT) has detected a variable high-energy source coinciding with the position of the x-ray binary and microquasar Cygnus X-3. Its identification with Cygnus X-3 is secured by the detection of its orbital period in gamma rays, as well as the correlation of the LAT flux with radio emission from the relativistic jets of Cygnus X-3. The gamma-ray emission probably originates from within the binary system, opening new areas in which to study the formation of relativistic jets.

Cyg X-3 (Cyg X-3) motivated to a large extent the development of high-energy gamma-ray astronomy. Detections of Cyg X-3 were reported in the 1970s

and early 1980s at energies of tens of mega-electron volts, tera-electron volts, peta-electron volts, and possibly exa-electron volts, generating considerable excitement as these identified

the system as a prime source of cosmic rays (1–3). However, the detections remained doubtful in part because observations in the late 1980s and in the 1990s by a more sensitive generation of ground-based telescopes failed to confirm the tera-electron volt and peta-electron volt detection of Cyg X-3 (4, 5), and also because both the COS-B satellite (6) and the CGRO-EGRET satellite (7) could not find emission from the source in the giga-electron volt range.

Here, we report the detection of a variable high-energy gamma-ray (100 MeV to 100 GeV) source that we identify with Cyg X-3 based on its location, the detection of a modulation of the gamma-ray flux at the orbital period of the binary system, and the gamma-ray variability correlated with the radio emission originating from the relativistic jets of Cyg X-3.

Cyg X-3 is a high-mass x-ray binary system (2) with a short orbital period of 4.8 hours (8), located in the Galactic plane at a distance of ~7 kpc from Earth (9). The nature of the compact object—neutron star or black hole—is still subject

to debate; however, the companion is known to be the Wolf-Rayet star V1521 Cyg (10). After its discovery in 1966 (11), Cyg X-3 was intensively observed over a wide range of wavelengths. It frequently becomes the brightest radio source among the binary systems with major flares because of its relativistic jets (12–14), which qualify the source as a microquasar. The variable emission observed from microquasars, ranging from radio frequencies up to a few hundreds of kiloelectron volts, is induced by the interplay between the stellar companion, the accretion flow, the optically thin electron corona, and the relativistic jets (15–17).

We present the results of the Fermi Large Area Telescope (LAT) observations of the Cygnus region between 4 August 2008 and 2 September 2009 [see the supporting online material (SOM) for details] (Fig. 1). The pulsar PSR J2032+4127 is located very close (~30 arc min) to Cyg X-3 and contributes substantially to the LAT photons at the location of Cyg X-3. However, because it exhibits relatively narrow pulses (18), it enabled us to select the LAT data using the off-pulse phase intervals of PSR J2032+4127, a procedure that preserves 80% of LAT live time. We then performed an unbinned maximum-likelihood spectral and spatial fitting analysis of a 15°-radius region centered on the position of Cyg X-3. Adding a source consistent with the location of Cyg X-3 in the fitting procedure improves the fit significantly. The overall significance of the LAT source at this position amounts to more than 29 standard deviations (29 σ). The position of the LAT source is right ascension (J2000) = 308.05° and declination (J2000) = 40.96° with a statistical uncertainty (95% confidence level) of 0.04°, which is consistent with the location of Cyg X-3.

We also constructed the LAT light curve on 4-day (Fig. 2) and 2-day time scales in order to highlight the gamma-ray variability of the LAT source, which is mostly dominated by two main active periods: 11 October to 20 December 2008 [modified Julian date (MJD) 54750 to 54820] and 8 June to 2 August 2009 (MJD 54990 to 55045). These phases of activity may be consistent with one or several flares, with the brightest flare possibly being observed at the end of each period. The peak flux can be as high as $\sim 2 \times 10^{-6}$ photons cm⁻² s⁻¹ above 100 MeV.

We have fitted the spectrum of Cyg X-3 in the two active periods with a simple power law using the data set created from the off-pulse phase intervals of PSR J2032+4127. We obtained a spectral index Γ of 2.70 ± 0.05 [statistical (stat)] ± 0.20 [systematic (syst)]. The systematic error was estimated by using different sets of models for the diffuse emission. In view of the soft

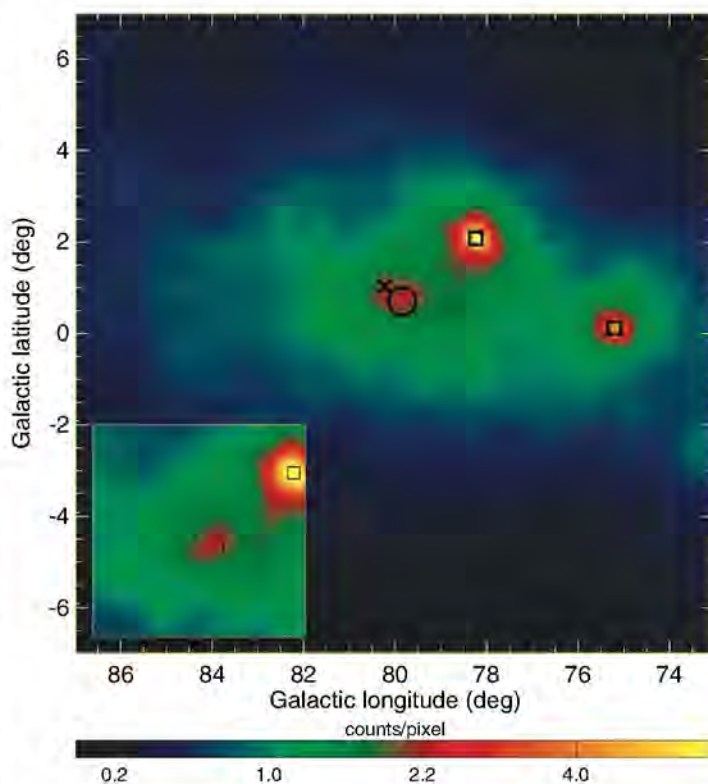
spectrum and the bright, complex, diffuse emission in this region, this spectral parameter may have a larger systematic uncertainty. However, for this preliminary spectral index, the average photon flux of the LAT source in the active periods is $[1.19 \pm 0.06$ (stat) ± 0.37 (syst)] $\times 10^{-6}$ photons cm⁻² s⁻¹ above 100 MeV, corresponding to an energy flux of $[4.0 \pm 0.3$ (stat) ± 1.3 (syst)] $\times 10^{-10}$ erg cm⁻² s⁻¹. This corresponds to a gamma-ray luminosity L_γ of $\sim 3 \times 10^{36}$ (distance/7 kpc)² erg s⁻¹, which is similar to what was originally reported by the SAS-2 satellite (19) in the early 1970s for Cyg X-3 in a similar energy range. The AGILE satellite also reported gamma-ray flares in the vicinity of Cyg X-3 at a similar epoch (20).

To securely identify the LAT source with Cyg X-3, we performed a timing analysis to search for the 4.8-hour orbital period (8) of Cyg X-3. For that purpose, we created a light curve for the LAT source using aperture photometry and 1000-s time bins. We extracted photons within a circular region with a radius of 1°, centered on Cyg X-3 in the energy range from 100 MeV to 100 GeV. We calculated the power spectra for both the entire light curve and also for only the active periods identified above (Fig. 2). Because 1000 s is shorter than the sky survey rocking period of Fermi, the exposure of Cyg X-3 varies considerably from time bin to time bin. We therefore weighted the contribution of data points to the power spectrum by their relative exposures. Although no orbital modulation is apparent when

the entire data set is used, two peaks, corresponding to the orbital period of Cyg X-3 and its second harmonic (in addition to low-frequency noise), stand out when the analysis is restricted to the two periods of enhanced emission (Fig. 3A). The nominal probability of a false detection for a period of arbitrary frequency is 3.6×10^{-5} , taking into account the number of trials. To investigate whether the modulation seen at the orbital period of Cyg X-3 could be an artifact, we also extracted light curves and calculated power spectra for all sources in the Fermi Bright Source List. In no case did we see significant modulation at the orbital period of Cyg X-3. A maximum-likelihood analysis of light curve photons (SOM) shows that the probability of finding a modulation as strong as the LAT signal with the known period of Cyg X-3 is $\sim 2.4 \times 10^{-9}$. This confirms that the LAT source displays the orbital period of Cyg X-3 and therefore secures its identification with Cyg X-3.

The orbital period of Cyg X-3 has been reported to show secular change (21). To obtain the predicted parameters of Cyg X-3 at the time of our LAT observations, we therefore used the parabolic ephemeris of Cyg X-3 (21) and derived a period of 0.199692441 (35) day and an epoch of minimum x-ray flux of MJD 54,856.693 (10). This is consistent with the period obtained— $P = 0.199655$ (46) day—from the maximum-likelihood analysis of the LAT light curve. We compared the LAT light curve from the active

Fig. 1. 200-MeV to 100-GeV Fermi/LAT gamma-ray counts map of a 14° × 14° region centered on the position of Cyg X-3 and corresponding to the active periods discussed in the text. The map (with no background subtraction) was adaptively smoothed by imposing a minimum signal-to-noise ratio of 15. It shows the region around Cyg X-3 encompassing emission from three bright gamma-ray pulsars (PSR J2021+4026, PSR J2021+3651, and PSR J2032+4127), as well as the strong Galactic diffuse emission of the Cygnus region. The locations of PSR J2021+4026 and PSR J2021+3651 are indicated by black squares, and PSR J2032+4127 is indicated by a cross. The position of Cyg X-3 is shown by the circle. (Inset)



Similar image for the central portion (4° × 4°), corresponding to the off-pulse data set from PSR J2032+4127 (which is not detected in the off-pulse data). A total of ~3400 counts were detected from the LAT gamma-ray source at the position of Cyg X-3.

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time ranges folded on the orbital period to the folded 1.5- to 12-keV x-ray light curve of Cyg X-3 from the Rossi X-ray Timing Explorer All-Sky Monitor (RXTE/ASM) (Fig. 3B). The folded x-ray and gamma-ray light curves have the same asymmetric shape, with a slow rise followed by a faster decay. However, a striking contrast is that the phase of LAT maximum flux is close to the phase of minimum x-ray count rate. Indeed, the LAT minimum trails the x-ray minimum by 0.3 to 0.4 in phase. Further observations will be needed to interpret the small (~ 0.1) shift in LAT maximum that occurs from the first to the second active period (SOM).

The soft x-ray light curves (1.5 to 12 keV) from the RXTE/ASM and hard x-ray light curves (15 to 50 keV) from the Swift/BAT satellite indicate that all LAT gamma-ray active periods from Cyg X-3 correspond to the soft x-ray state of the source (fig. S2). Cyg X-3 is known to flare in radio in the soft state (22), with associated relativistic plasma ejection events (12–14). We have therefore compiled the radio data from our regular monitoring with the AMI telescope and the OVRO 40-m tele-

scope at 15 GHz. Inspection of the LAT and radio light curves of Cyg X-3 (Fig. 2) indicates that the LAT active periods occur close to radio flares. To quantify the relation between the gamma-ray and radio emission, we have calculated the discrete cross-correlation function normalized only by the sample variance (fig. S3). We find a positive correlation between the two wave bands. The overall correlation is dominated by the strong radio and gamma-ray flare around MJD 54810. The peak in the cross-correlation is significant at more than $\sim 3\sigma$ (the exact value depending on the assumed noise properties of the LAT data on Cyg X-3). The lag of the radio light curve to the gamma rays is not well constrained; a bootstrapping technique shows that it is 5 ± 7 days.

The detection of Cyg X-3 by the LAT during periods of relativistic ejection events is important for linking and understanding the different components of an accreting binary system (the wind of the Wolf-Rayet star, the compact object, and the accretion disk, corona, and relativistic jets). A variable hard tail above 30 keV with a power law index $\Gamma \approx 2$ is present in some in-

stances (the ultrasoft state) of the soft x-ray state associated with the onset of radio flaring episodes (22). The tail has been reported to extend up to several hundred kilo-electron volts (23). The Fermi flux is compatible with the extrapolation of this tail to 100 MeV but has a steeper spectrum, suggesting that we are seeing the high-energy end of a spectral component peaking in soft gamma rays.

The hard x-ray tail is typically modeled as being due to the Compton upscattering of photons from a soft photon source by high-energy electrons in a corona (possibly the base of the jets), although the physical processes responsible for the electrons' acceleration are not yet known (22, 23). It is likely that the gamma-ray emission is due to the same process; however, this is only possible if the emission region is not too close to the accretion disk, or it would be absorbed by pair production on the soft x-ray photons (24). On the other hand, the gamma-ray modulation suggests (25) that the emission region size and location are bounded by the orbital separation $a \approx 2.5 \times 10^{11}$ cm.

With the emitting region located away from the accretion disk but within the system, the companion star provides the dominant radiation field at the location of the electrons. Electrons with energies of a few giga-electron volts can then inverse Compton-scatter the ultraviolet (UV) photons from the star [surface temperature (T_*) $\approx 10^5$ K] to gamma rays. The stellar radiation field is not isotropic for the electrons, so the upscattered emission also will be anisotropic, causing a modulation of the gamma-ray emission. Assuming that the location of the electrons is in phase with the compact object (as expected for jets perpendicular to the orbital plane), maximum gamma-ray emission occurs when the electrons are seen behind the Wolf-Rayet star; that is, at superior conjunction when the energetic electrons directed toward Earth suffer head-on collision with the stellar UV photons (26). This corresponds to minimum x-ray emission if the x-ray modulation is due to Compton scattering in the companion's wind as is usually presumed (27). Infrared studies also support this phasing of the orbit (28). The phase shift of the gamma-ray minimum (fig. S1) from $\phi = 0$ might be associated with misalignment of the axis of the jets.

The inverse Compton energy-loss time scale $t_{ic} \approx 1/E_{GeV} s$ is short compared to the escape time scale $t_{esc} = a/c \approx 8$ s, meaning that this radiative process is quite efficient. The maximum observed gamma-ray luminosity L_γ is $5 \times 10^{36} d_{7kpc}^2 \text{ erg s}^{-1}$ above 100 MeV (for a spectral index of $\Gamma = 2.7$), which is $\leq 10\%$ of the x-ray luminosity, suggesting that only a minor fraction of the accretion power is released via particle acceleration. This contrasts with the few other binaries (29) that are confirmed as high-energy gamma-ray emitters. In these, the dominance of gamma rays in the nonstellar radiative output, the weakness of the x-ray emission

Fig. 2. Gamma-ray light curve in two parts (top and bottom panels) with a likelihood analysis window of 4 days of the LAT source at the location of Cyg X-3. The LAT fluxes (left axis) are expressed in units of $10^{-6} \text{ photons cm}^{-2} \text{ s}^{-1}$ above 100 MeV. A power law shape with spectral index of 2.7 is assumed for Cyg X-3 in the analysis. The parameters of the components for the model of the diffuse emission were held fixed to the values obtained in the global fit, with the exception of the normalization of the Galactic diffuse component that was left free. The vertical dashed lines illustrate the active periods discussed in the text. The bottom horizontal dotted line in each panel indicates the average gamma-ray flux at the location of Cyg X-3 outside the active periods. In addition, at the top of each panel, we show the Cyg X-3 15-GHz radio flux (from the AMI and OVRO 40-m radio telescopes) as a function of time (axis on the right). An offset of 0.124 Jy has been removed from the OVRO data to account for the effect of extended nearby sources that are resolved out by the AMI interferometer. A horizontal dotted line at 0 Jy is also plotted for reference. Several radio flares were detected during the LAT active periods.

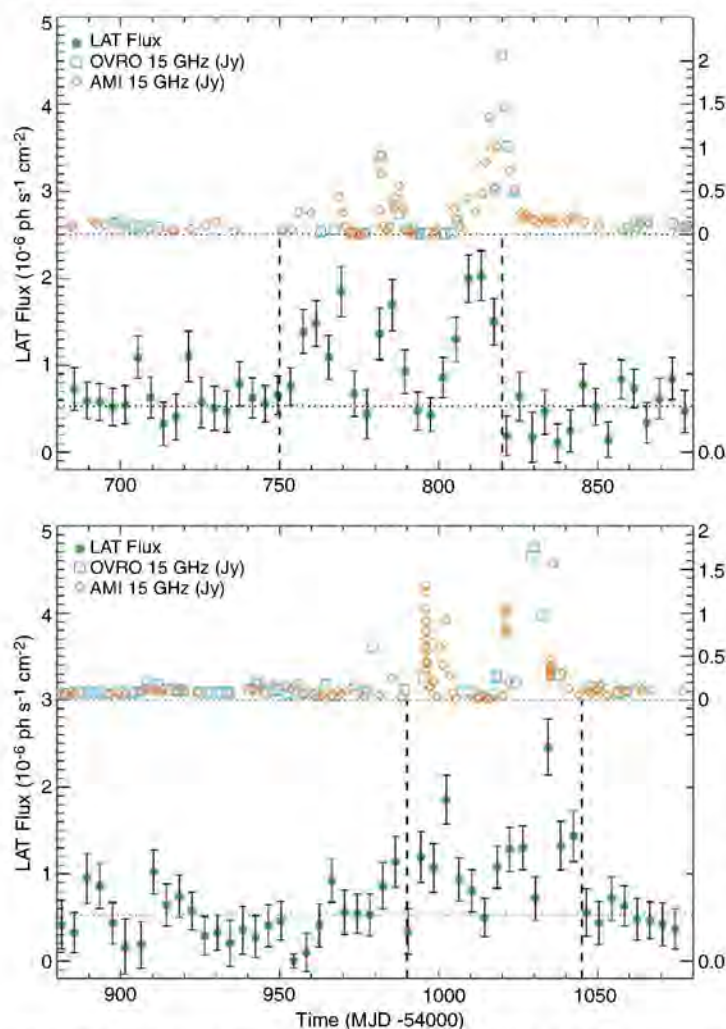
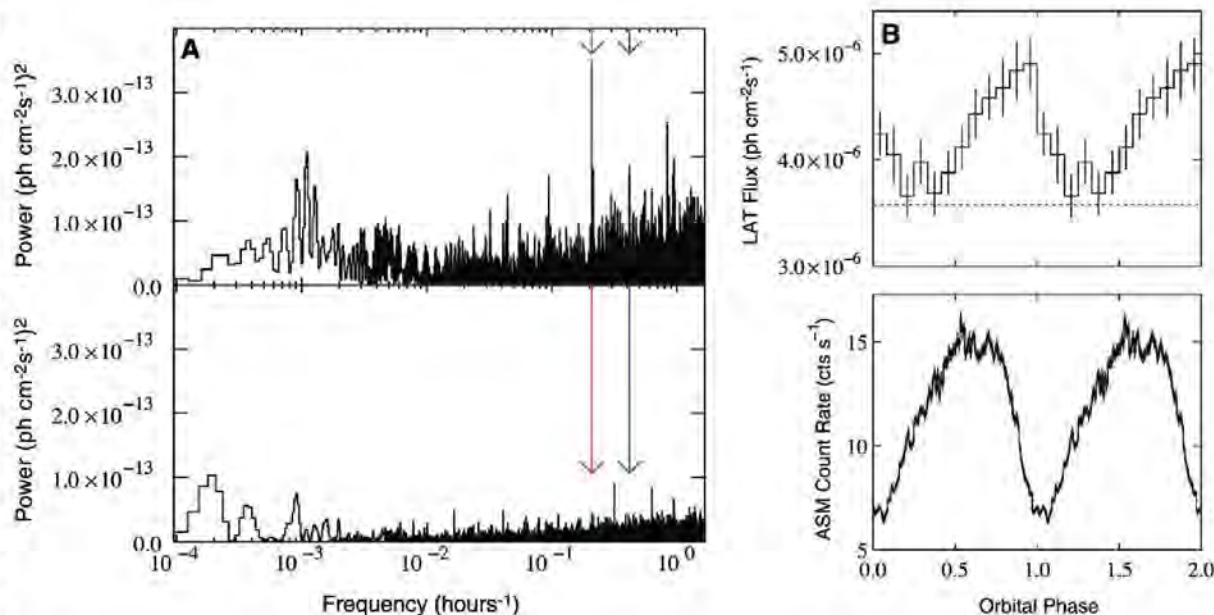


Fig. 3. Power spectra and folded light curves of the LAT source at the location of Cyg X-3. **(A)** (Bottom) Power spectrum of the entire LAT light curve. (Top) Power spectrum of the identified active periods of the LAT source. The red and blue arrows indicate the frequencies that correspond to the orbital period and the second harmonic of the orbital period, respectively. **(B)** (Top) Light curve of Cyg X-3 during times identified as the active intervals [same as top panel in (A)] of the LAT source folded on the orbital period of the system. The data points were again weighted by their exposures. The dashed line shows the emission level (including diffuse emission) obtained by measuring the flux in the 1° aperture outside the active periods of Cyg X-3. In our definition, the compact object is directly behind the



($<10^{34}$ erg s $^{-1}$), and the lack of accretion signatures suggest a compact pulsar wind (30).

Interpreted as the propagation time of relativistic ejecta, the 5-day delay (still uncertain, however) between the onset of gamma-ray and radio flaring implies that the radio emission occurs at $\approx 1.3 \times 10^{16}$ cm [≈ 900 astronomical units ≈ 125 milli-arc sec (mas)] away from the system. Indeed, radio emission during flares is known to occur farther out in the jet on scales of hundreds of astronomical units (at a distance from the core of few tens of milli-arc seconds), with measured projected expansion velocities around 10 mas/day [0.5 to 0.6 c (14)].

Unique characteristics of Cyg X-3 as compared to all other microquasars are the very tight orbit and very strong Wolf-Rayet wind. With a wind mass loss $\sim 10^{-5}$ solar mass year $^{-1}$ and a wind velocity ~ 1000 km s $^{-1}$ (10), the densities (hereafter n_{13} in units of 10^{13} cm $^{-3}$) reach 10^{13} cm $^{-3}$ at the location of the compact object, which is orders of magnitude more than in all other systems. Thus, although we have discussed a leptonic origin for the giga-electron volt photons, if cosmic ray protons are also accelerated in the source, they could have substantial interaction with the nuclei in the stellar wind, producing charged and neutral pions that would decay into gamma rays and neutrinos. The time scale for proton-proton interaction is $t_{pp} \approx 100 n_{13}^{-1} s > t_{esc}$. Assuming a 10% transfer efficiency of the proton energy into secondary particles, the required proton luminosity is $\sim 10(t_{pp}/t_{esc})L_\gamma$. Thus, the non-thermal energy would be larger than or (if the protons are confined) comparable to the x-ray luminosity. If this scenario is correct, something that the observation of neutrinos from this source would confirm, then the dense environment of Cyg X-3 could provide an opportunity

to study the high-energy proton content in microquasars and their contribution to cosmic ray protons.

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Figs. S1 to S3

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Organic Nonvolatile Memory Transistors for Flexible Sensor Arrays

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Using organic transistors with a floating gate embedded in hybrid dielectrics that comprise a 2-nanometer-thick molecular self-assembled monolayer and a 4-nanometer-thick plasma-grown metal oxide, we have realized nonvolatile memory arrays on flexible plastic substrates. The small thickness of the dielectrics allows very small program and erase voltages (≤ 6 volts) to produce a large, nonvolatile, reversible threshold-voltage shift. The transistors endure more than 1000 program and erase cycles, which is within two orders of magnitude of silicon-based floating-gate transistors widely employed in flash memory. By integrating a flexible array of organic floating-gate transistors with a pressure-sensitive rubber sheet, we have realized a sensor matrix that detects the spatial distribution of applied mechanical pressure and stores the analog sensor input as a two-dimensional image over long periods of time.

Electronic devices are traditionally fabricated using inorganic semiconductors, rigid substrates, and high-temperature manufacturing methods. In contrast, organic semiconductors can be processed at low temperatures and on large-area polymeric substrates. This has allowed for the development of a variety of electronic devices on

flexible plastic substrates, including solar cells (1), light-emitting diode displays (2), field-effect transistors (3), transponders (4), sensors (5), actuators (6), and nonvolatile memory transistors (7–11). Nonvolatile memory transistors are potentially useful to individualize radio-frequency transponders or to store data obtained by large-area sensor arrays

for later read-out. Most of the organic memory transistors reported to date exploit the electric field-induced remnant polarization in ferroelectric polymer films (7–11). A considerable limitation of ferroelectric polymer memory transistors is that the coercive field required to reverse the macroscopic polarization increases with decreasing film thickness (12), which makes it difficult to obtain a large enough memory window with program and erase voltages below about 20 V. Also, due to the substantial surface roughness of the ferroelectric polymer films, the carrier field-effect mobility in these transistors is usually quite low (<0.1 cm²/Vs).

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A floating-gate transistor is a field-effect transistor with two gate electrodes. In addition to the control gate, similar to that in a regular transistor, it has a floating gate embedded in the gate dielectric. When the dielectric is thin enough, electronic charge can be brought onto the floating gate by quantum tunneling or thermal emission when a large enough program voltage is applied between

the control gate and the source contact. Charging the floating gate changes the transistor's threshold voltage, because the charge on the floating gate partially screens the electric field between the control gate and the semiconductor. This threshold voltage shift can be detected by measuring the drain current at a certain gate-source voltage. Because the floating gate is completely isolated by the di-

electric, charge stored on the floating gate remains there without the need for any applied voltage (nonvolatile memory). To erase the memory, a voltage of opposite polarity is applied, discharging the floating gate through the dielectric.

In silicon-based floating-gate transistors, the dielectric is a silicon dioxide layer with a thickness of a few nanometers. The exact thickness is a

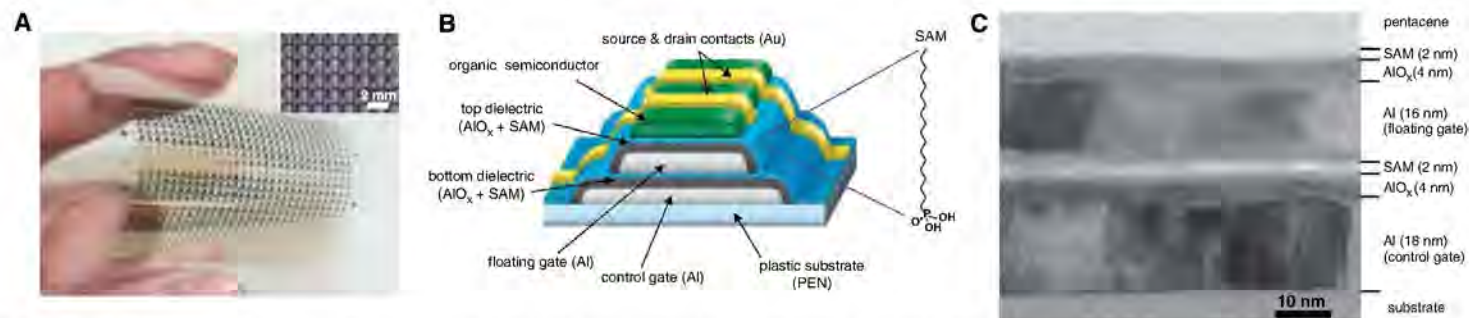


Fig. 1. (A) Photograph of an organic floating-gate transistor sheet comprising 26 by 26 memory cells. The array has an effective area of 50 by 50 mm². The inset shows a magnified image of the array. (B) Schematic cross section of the floating-gate transistors. The substrate is flexible PEN. The control and floating gates are 20-nm-thick layers of evaporated aluminum. The top and bottom

dielectrics are each a combination of a 4-nm-thick layer of AlO_x and a 2-nm-thick SAM. The organic semiconductor is a 50-nm-thick layer of pentacene, and the source and drain contacts are 50-nm-thick layers of evaporated gold. (C) Cross-sectional TEM images of a flexible floating-gate transistor. The specimen was prepared using a focused ion beam and imaged by TEM (300 kV).

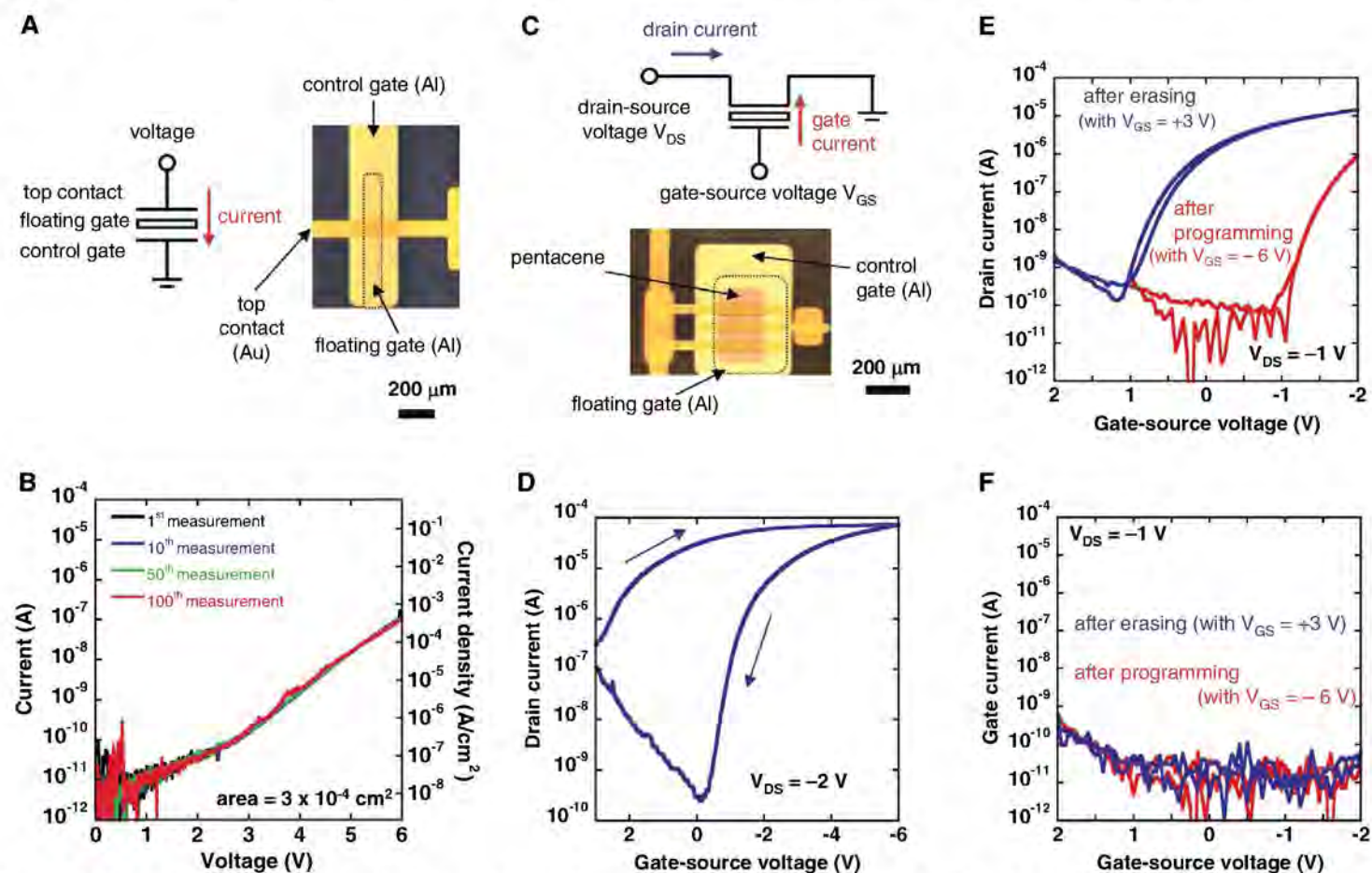


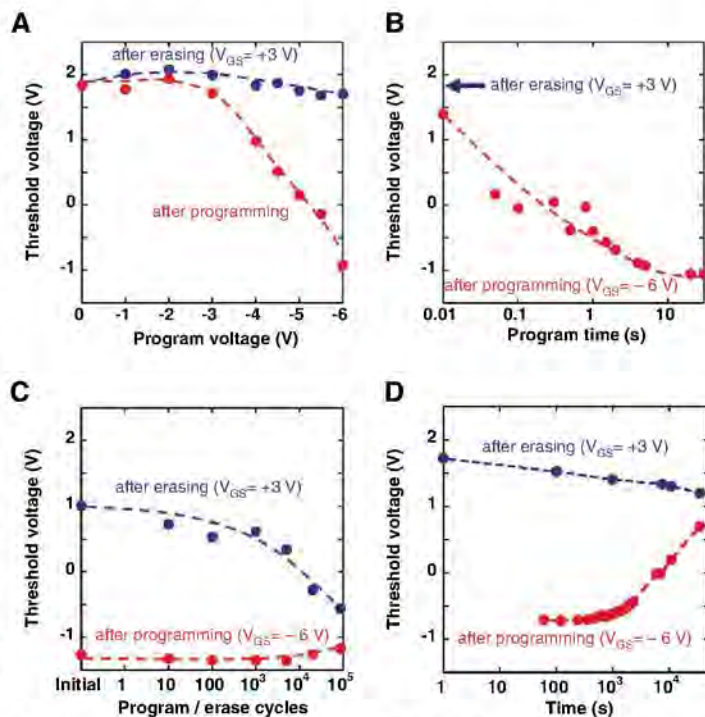
Fig. 2. Static electrical performance of the floating-gate memory devices. (A) Photograph of a floating-gate capacitor used for characterizing the voltage-dependent current through the AlO_x/SAM dielectrics. (B) Current through the AlO_x/SAM dielectrics as a function of applied voltage. Up to 6 V, the current does not cause irreversible changes in the dielectrics. (C) Photograph of a pentacene floating-gate memory transistor. (D) Drain current as a function of the voltage applied between control gate and source contact. When the

applied voltages are -6 V and +3 V, the floating gate is charged and discharged, causing a threshold-voltage shift and hysteresis in the current-voltage characteristics. (E) Read-out operation performed on a floating gate transistor after programming and after erasing for 1 s. The threshold-voltage shift induced by the program and erase operations is clearly observed. (F) Gate current of a floating-gate transistor measured during read-out. For read-out voltages up to ±2 V, the gate current reaches about 100 pA.

compromise between the voltage required to bring enough charge onto the floating gate and the charge leakage from the floating gate, which sets an upper limit on the retention time. Silicon-based floating-gate transistors typically have program and erase

voltages of 10 to 20 V (13, 14) and retention times of several years. Because the defect density in the thin dielectric increases with every program and erase operation, the endurance is usually limited to $\sim 10^6$ program and erase cycles (14).

Fig. 3. Threshold voltage of organic floating-gate transistors. (A) Threshold-voltage shift as a function of program voltage. The program voltage is applied for 1 s. At the maximum program voltage of -6 V, a threshold-voltage shift of about 2.5 V is obtained. The threshold voltage after erasing with $+3$ V is independent of the program voltage. (B) Threshold-voltage shift as a function of the duration of the program pulse. The program voltage is -6 V. (C) Endurance of the memory transistors. The threshold-voltage window is greater than 2 V after 10^3 program and erase (-6 to $+3$ V) cycles and greater than 1 V after 10^4 cycles. (D) Retention characteristics of the memory transistors. The threshold voltage window is greater than 2 V after 10^3 s and greater than 1 V after 10^4 s. For each data point, the threshold voltage was obtained by measuring the complete transfer characteristics [sweeping gate-source voltage (V_{GS}) from $+2$ to -2 V; drain-source voltage (V_{DS}) = -1 V].



Although silicon floating-gate transistors are excellent for high-density data storage, flexible organic floating-gate transistors are potentially useful for large-area sensors and actuators with integrated nonvolatile memory capability. There are very few reports of organic floating-gate transistors, most of which use rigid substrates, thick dielectrics (10 to 50 nm) and large program and erase voltages (15 to 50 V), and none of which have demonstrated integration of these transistors into memory arrays (15–18). The main challenge in reducing the program and erase voltages and in demonstrating high yield and sufficient uniformity in large-area memory arrays on flexible plastic substrates is to develop a dielectric that can be prepared below the glass transition temperature of plastic film ($<150^\circ\text{C}$) and which combines small film thickness with good reproducibility and small defect density. By taking advantage of the excellent properties of a low-temperature hybrid dielectric based on a thin metal oxide and a self-assembled monolayer (SAM), we have developed flexible floating-gate transistors with small program and erase voltages (-6 V to $+3$ V). Figure 1A shows a photograph of a plastic sheet with 676 organic floating-gate transistors arranged in a 26 by 26 array on a $125\text{-}\mu\text{m}$ -thick plastic film. The schematic device cross section is shown in Fig. 1B; the fabrication process is outlined in the Supporting Online Material. The two dielectrics that isolate the floating gate from the control gate and the organic semiconductor are a combination of a thin aluminum oxide (AlO_x) layer grown in an oxygen plasma at room temperature and an alkyl-phosphonic acid SAM

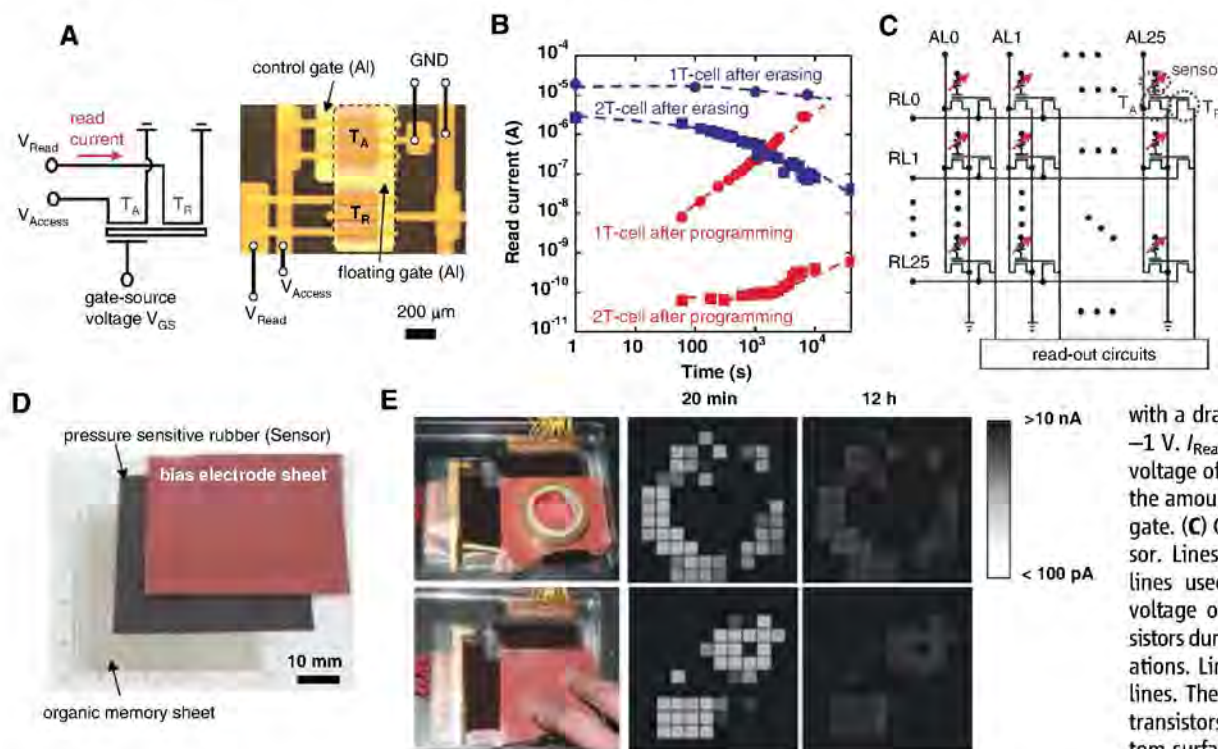


Fig. 4. Flexible pressure-sensor array. (A) Schematic and photograph of a two-transistor (2T) memory cell in which an access transistor (T_A) and a read-out transistor (T_R) share a large floating gate. (B) Retention characteristics of a 2T cell in comparison with that of a 1T cell. The read current (I_{Read}) is the drain current of T_R measured

with a drain-source voltage (V_{Read}) of -1 V. I_{Read} depends on the threshold voltage of the transistors and thus on the amount of charge on the floating gate. (C) Circuit schematic of the sensor. Lines marked AL are the access lines used to apply a drain-source voltage of -2 V to the access transistors during program and erase operations. Lines marked RL are the read lines. The control gates of all access transistors are connected to the bottom surface of the pressure-sensitive rubber sheet. (D) Photograph of the

three individual sheets before lamination. Bottom, $125\text{-}\mu\text{m}$ -thick PEN sheet with 676 2T memory cells; center, $500\text{-}\mu\text{m}$ -thick pressure-sensitive rubber sheet; top, $125\text{-}\mu\text{m}$ -thick PEN sheet with copper electrode. (E) Demonstration of the sensor array. The spatial distribution of mechanical pressure applied using two different objects is stored in the organic floating-gate transistors and can be retrieved even after the pressure and voltages have been removed.

prepared from solution at room temperature, with a total thickness of about 6 nm and a capacitance of 0.6 to 0.65 $\mu\text{F}/\text{cm}^2$ (19–22). The semiconductor is a thin layer of vacuum-deposited pentacene.

Figure 1C shows a cross-sectional electron microscopy (EM) image of a completed device. The specimen was prepared using a focused ion beam (FIB) (FB-2100, Hitachi High-Technologies Corp., Tokyo, Japan) and imaged by transmission electron microscopy (TEM) (HF-3300 Cold-FE TEM, 300 kV, Hitachi High-Technologies Corp.). The control gate, the floating gate, and the two dielectric layers can be clearly distinguished in the TEM image. Perhaps most notably, the 2-nm-thick organic SAM of the bottom dielectric that separates the control gate from the floating gate is clearly resolved. The TEM image confirms the structure of the organic floating-gate transistors.

To characterize the electric current through the dielectrics during program and erase operations, floating-gate capacitors were prepared without the semiconductor (Fig. 2A). Figure 2B shows that when the voltage between the control gate and the top contact increases, the current also increases, reaching 0.5 mA/cm^2 at 6 V. This measurement was repeated 100 times without any changes in the current-voltage curves, showing that the dielectric can sustain this current. Beyond 6.3 V, however, an irreversible increase in current was observed, indicating damage to the dielectric (fig. S3A).

Figure 2C shows a photograph of a pentacene floating-gate memory transistor. Initially, the threshold voltage (V_{th}) is between +1 and +2 V (Figs. 2 and 3). For programming, a voltage of –6 V is applied between the control gate and the source contact. This creates a gate current of 1 μA (fig. S3B), which charges the floating gate and shifts V_{th} to –1 V. To erase, a voltage of +3 V is applied to discharge the floating gate and recover the initial threshold voltage. Cycling the gate-source voltage between +3 V and –6 V produces the hysteresis seen in Fig. 2D. The exact V_{th} shift depends on the voltage and duration of the program pulse (Fig. 3, A and B, and figs. S4 and S5). To read the stored information, V_{th} is determined, for example, by measuring the drain current as a function of gate-source voltage. Figure 2E shows the result of two read-out operations, one on a transistor programmed at –6 V, the other on a transistor erased at +3 V. The threshold voltage window and the device-to-device uniformity (fig. S6) are sufficient for unambiguous read-out. Figure 2F shows that if read-out is performed with a gate-source voltage of ± 2 V, the gate current reaches 100 pA. This causes a small portion of the stored charge to be lost and V_{th} to shift slightly during each read-out operation (destructive read-out) (fig. S7, A and B). In contrast, if read-out is performed with a gate-source voltage of 0 V, there is no charge loss and no V_{th} shift (nondestructive read-out) (fig. S7C).

Modern silicon-based flash memory is typically rated for 10^6 program and erase cycles (14), after which the memory characteristics degrade. To evaluate the endurance of the organic memory transistors, a device was subjected to 10^5

program and erase cycles (–6 V to +3 V, 1 Hz). The results in Fig. 3C and in fig. S8 show that the threshold voltage window is constant at 2 V up to 10^3 cycles. Beyond that, the positive threshold voltage shift during the erase operation becomes smaller, so the initial threshold voltage of +1 V is no longer recovered and the threshold voltage window becomes smaller. Nonetheless, after 10^4 cycles, the threshold voltage window is still 1 V, sufficient for unambiguous read-out. After 10^5 cycles, the threshold voltage window is 0.5 V. Interestingly, the initial threshold voltage of +1 V can be recovered by annealing the transistors at 140°C in dry nitrogen, which suggests that the endurance is limited not by irreversible structural damage but by reversible carrier trapping.

Unlike dynamic random access memory (DRAM), nonvolatile memories retain information in the absence of external voltages. In floating-gate transistors, this requires that the charge on the floating gate is prevented from leaking away through the dielectric. Data retention experiments on an individual pentacene device (shown in Fig. 3D) suggest that the retention time is only a few hours (threshold voltage window is 1.1 V after 3 hours, 0.6 V after 12 hours). However, most of the charge loss in this case occurred during read-out. To improve retention, we have therefore designed and fabricated a two-transistor memory cell in which an access transistor (with control gate) and a read-out transistor (without control gate) share a floating gate with an area of 400 by 1000 μm^2 (Fig. 4A). Read-out is performed with a drain-source voltage of –1 V applied to the read-out transistor for 0.1 s or less, with no voltage applied on the control gate. This resulted in a significant improvement in retention time: After 3 hours, the read-current ratio is less than 2 for the one-transistor cell, but 2×10^2 for the two-transistor cell. After 12 hours, the two-transistor cell still has a usable large read-current ratio of 10^2 (Fig. 4B).

To demonstrate the potential of organic floating-gate transistors and the two-transistor memory cell design, we fabricated a large-area flexible sensor that measures the spatial distribution of mechanical pressure applied to it and retains this data for more than 12 hours after the pressure and voltages have been removed. The sensor was fabricated by laminating three sheets: a polyethylene naphthalate (PEN) sheet with 676 two-transistor memory cells arranged in a 26 by 26 array, a pressure-sensitive rubber sheet, and a PEN sheet with a copper electrode. The circuit schematic is shown in Fig. 4C, a photograph of the three individual sheets before lamination is shown in Fig. 4D, photographs of the memory array are shown in fig. S10A, and a cross section of the laminated stack is shown in fig. S10B. The control gates of all 676 memory cells are connected to the bottom surface of the rubber sheet, and the top surface of the rubber sheet is in contact with the copper electrode. When mechanical pressure is applied to the rubber sheet, the electrical resistance between the rubber's top and bottom surfaces decreases (fig. S10C). By

applying a program voltage to the copper electrode and an access voltage to all memory cells, the copper electrode supplies the program voltage to the floating-gate transistors in those positions where pressure is applied, and the pressure distribution is stored in the memory array.

Figure 4E shows a demonstration of the sensor. Pressure was applied using two different objects: a roll of tape and two fingers. The stored information was read out after 20 min and again after 12 hours with a multichannel drive system. As can be seen, the contrast between the programmed cells and the background deteriorates over time due to charge loss (Fig. 4B), but even 12 hours after removing the mechanical pressure and the electric voltages, the stored information showing the spatial distribution of the applied pressure was still successfully recovered.

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Gigahertz Dynamics of a Strongly Driven Single Quantum Spin

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Two-level systems are at the core of numerous real-world technologies such as magnetic resonance imaging and atomic clocks. Coherent control of the state is achieved with an oscillating field that drives dynamics at a rate determined by its amplitude. As the strength of the field is increased, a different regime emerges where linear scaling of the manipulation rate breaks down and complex dynamics are expected. By calibrating the spin rotation with an adiabatic passage, we have measured the room-temperature “strong-driving” dynamics of a single nitrogen vacancy center in diamond. With an adiabatic passage to calibrate the spin rotation, we observed dynamics on sub-nanosecond time scales. Contrary to conventional thinking, this breakdown of the rotating wave approximation provides opportunities for time-optimal quantum control of a single spin.

Two-level quantum systems have proven a fruitful ground for many emerging technologies. Recently, there have been a growing number of solid-state implementations of individually addressable two-level systems (1) for quantum information processing as well as fundamental studies of a single quantum object. These include superconducting qubits (2, 3), optically and electrically controlled single spins in quantum dots (4–6), individual charges in quantum dots (7), donors in Si (8, 9), and nitrogen vacancy (NV) centers in diamond (10–13). A critical issue for quantum information processing is the rate at which the two-level state can be manipulated compared to its coherence time because of the practical need for fault-tolerance (14). This necessitates efforts to both extend the coherence time and increase the manipulation rate of two-level systems.

Coherent manipulation of two-level systems is commonly achieved by applying a weak oscillating field at a frequency resonant with the energy level splitting (15). These driven two-level dynamics are described in terms of a pseudospin-1/2 with a Larmor field, H_0 , responding to an oscillating field that is the sum of two fields that rotate in opposite directions. One of the fields rotates with the spin during its precession and applies a constant torque. This corotating field produces Rabi oscillations between the two energy eigenstates because of spin precession around it. The counterrotating field has negligible impact on the spin dynamics provided that it is small compared with H_0 because the direction of the torque it applies on the spin varies rapidly in time and therefore averages out. This argument forms the basis of the rotating wave approximation (15) and is the cornerstone of both theory and experiment for nearly all two-level resonance phenomena. In the “strong-driving” regime, where the rotating fields have amplitudes roughly equal to H_0 , the spin dynamics are predicted (16, 17) to become highly anharmonic as the co- and counter-

rotating fields generate dynamics on the same time scale as the Larmor precession. These dynamics are not chaotic, but they are also not a small modulation of sinusoidal Rabi oscillations seen in classical systems (18). We experimentally examined these dynamics in a single quantum spin at room temperature by using an NV center in diamond driven by an oscillating field through an on-chip waveguide. This regime has been of strong theoretical interest on fundamental grounds (16, 17) and in the context of optimal control theory (19–21). Rather than avoiding the effects of the counterrotating field, we studied its influence on a single spin where the dynamics can be transparently interpreted. We found that it can be exploited to produce sub-nanosecond spin flips. Lastly, to understand these results more deeply, we theoretically modeled our experiment and captured the important details.

An NV center is a spin-1 diamond lattice defect composed of a substitutional nitrogen atom with an adjacent lattice vacancy. In this room-temperature experiment, we operated at a static external field of 850 G so that the energy splitting of the $m_s = 0$ and $m_s = -1$ spin states was 0.49 GHz (Fig. 1A). This splitting is the Larmor field, H_0 , given in frequency units because it determines the natural precession rate of the spin. In addition, the $m_s = 0$ to $+1$ transition is strongly Zeeman split from the $m_s = 0$ to -1 transition by ~ 4.8 GHz, so the NV center dynamics should remain that of a pseudospin-1/2 at the strongest driving field. Spin-selective relaxation from the $m_s = \pm 1$ state via an alternative pathway causes the visible photoluminescence intensity, I_{PL} , to be linearly dependent on the population P of the $m_s = 0$ spin level (22, 23). Because the relaxation end-point is $m_s = 0$, illumination initializes the spin into this state (Fig. 1A). In our time-domain measurements, we first optically initialized the NV center and turned the laser off. A pulsed, microwave-frequency driving field was then applied, and lastly the laser and the photon detector were turned on for readout (Fig. 1B).

The microwave driving field was applied via high-bandwidth, short-terminated coplanar waveguides fabricated on the surface of a [001]-oriented type Ib diamond using optical lithography (Fig. 1, C and D). For weak driving, when we apply a pulse of microwave radiation, P will evolve from the initialized state ($P = 1$) according to Rabi's formula (15):

$$P = 1 - \frac{H_1^2}{H_1^2 + \Delta H_0^2} \sin^2\left(\pi \sqrt{H_1^2 + \Delta H_0^2} t\right) \quad (1)$$

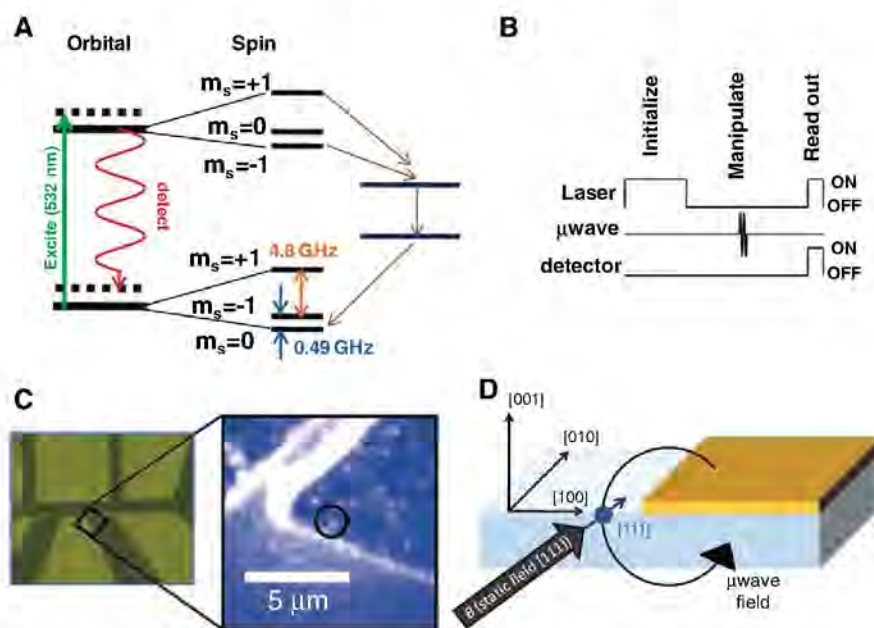


Fig. 1. (A) Simplified level structure of an NV center including orbital and spin levels at $B = 850$ G. Spin-selective relaxation from $m_s = \pm 1$ into $m_s = 0$ via an alternative pathway enables read out of the spin state via photoluminescence and optical pumping into the spin ground state. (B) Measurement duty cycle. (C) (Left) Optical micrograph of the terminated section of the coplanar waveguide on diamond. (Right) Spatial photoluminescence map of the diamond substrate near the waveguide termination. The small white spots are NV centers. The NV center that we describe is circled. (D) Experimental geometry.

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where ΔH_0 is the detuning from the transition frequency H_0 , H_1 is the amplitude of the rotating fields, and t is time. We used microwave pulses resonant with the transition so that $\Delta H_0 \sim 0$. In the experiment, we did not directly monitor the continuous time evolution of P ; instead we sampled the evolution by applying microwave pulses of progressively longer duration and then measured P (Fig. 1B). For weak driving, we expected sinusoidal oscillations between I_{PL} for the $m_s = 0$ state ($P = 1$) and I_{PL} for the $m_s = -1$ state ($P = 0$) with an oscillation frequency given by H_1 . The I_{PL} values of these eigenstates were calibrated by measuring I_{PL} after optical initialization into $m_s = 0$ and after an adiabatic passage into $m_s = -1$ (24). Figure 2A shows a plot of the Rabi frequency measured as a function of microwave power, which follows a square root trend (solid line). Figure 2B shows five Rabi measurements represented by the points in Fig. 2A.

The experimental Rabi oscillations at the smallest driving field, $H_1 = 29$ MHz, are smooth and sinusoidal, in agreement with Eq. 1. As we drove the spin harder, anharmonic components and steps emerged. At 223 MHz, there is an apparent "stalling" of P for a few ranges of pulse widths. Lastly, at $H_1 = 440$ MHz ($H_0/H_1 \sim 1.1$) the driving is strongly nonlinear; at some points, the spin was rotating much faster than expected from the corotating component of driving field alone. In this regime, the co- and counterrotating fields can either act in concert to rapidly change P or act against one another to keep P constant for an extended period. For example at $t \sim 7.5$ ns and $H_1 = 440$ MHz, there is a complete spin flip from pulses whose widths nominally differ by less than 0.5 ns (arrow, Fig. 2B).

To gain more insight into these dynamics, we performed numerical simulations by using pulses measured from the experiment with no free pa-

rameters (24). Figure 2C shows the simulated time evolution of P during the microwave pulse with a nominal duration of 10 ns, plotted below on the same time axis. The simulation shows dynamics qualitatively similar to those in the experiment, including stalling and periods of fast spin rotation. The simulations also show the population of $m_s = +1$ is at or below 10^{-5} even for the strongest driving field, confirming our assumption that the behavior is purely that of a pseudospin-1/2.

Simulations were performed separately for each experimental pulse to determine the final state populations and account for transient effects in the experiment. Strong-driving pulses are short and intense, so the leading and trailing edges unavoidably make up a substantial fraction of the pulse and have more influence than in standard spin resonance experiments. The simulated final state populations, plotted in Fig. 2D next to the experimental data, are in excellent qualitative

Fig. 2. (A) Experimental Rabi frequency as a function of microwave power at a static magnetic field of 850 G applied along the NV symmetry axis (H_0 for the $m_s = 0$ to -1 transition is 0.49 GHz). The Rabi field is calculated from the power for the open circles because the data do not fit to a sinusoid. (B) Experimental Rabi oscillations measured by collecting I_{PL} as a function of pulse width for five different driving fields, H_1 . The dashed red lines correspond to an $m_s = 0$ population P of 1 (upper) and 0 (lower). (C) P as a function of time for simulated spin dynamics using the microwave pulse from the experiment. The pulse is plotted on the same scale and normalized to $H_1 = 440$ MHz in the simulation. (D) Simulated final population of the $m_s = 0$ level as a function of pulse width for the same driving fields as in (B).

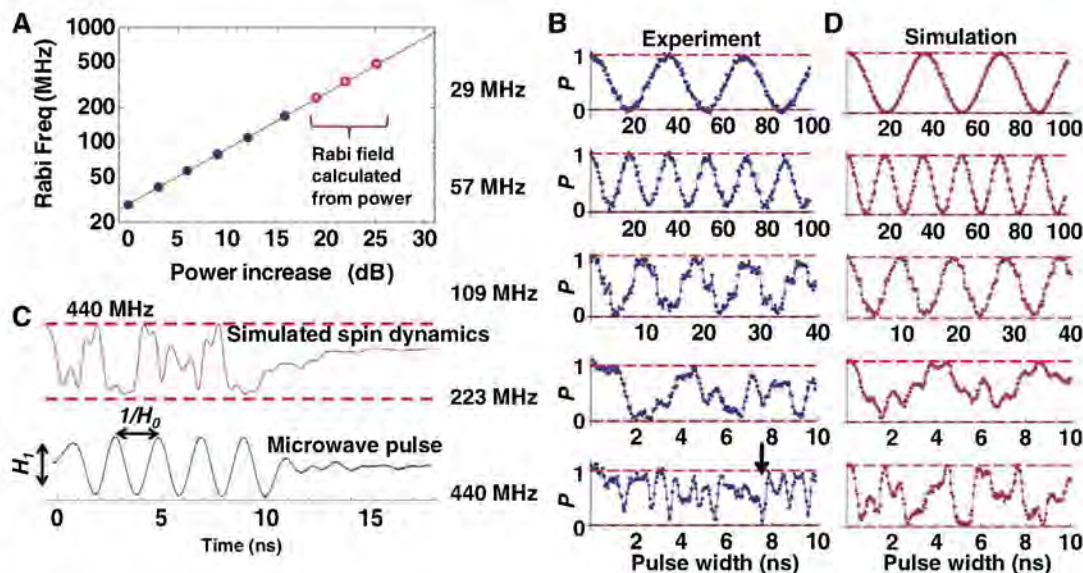
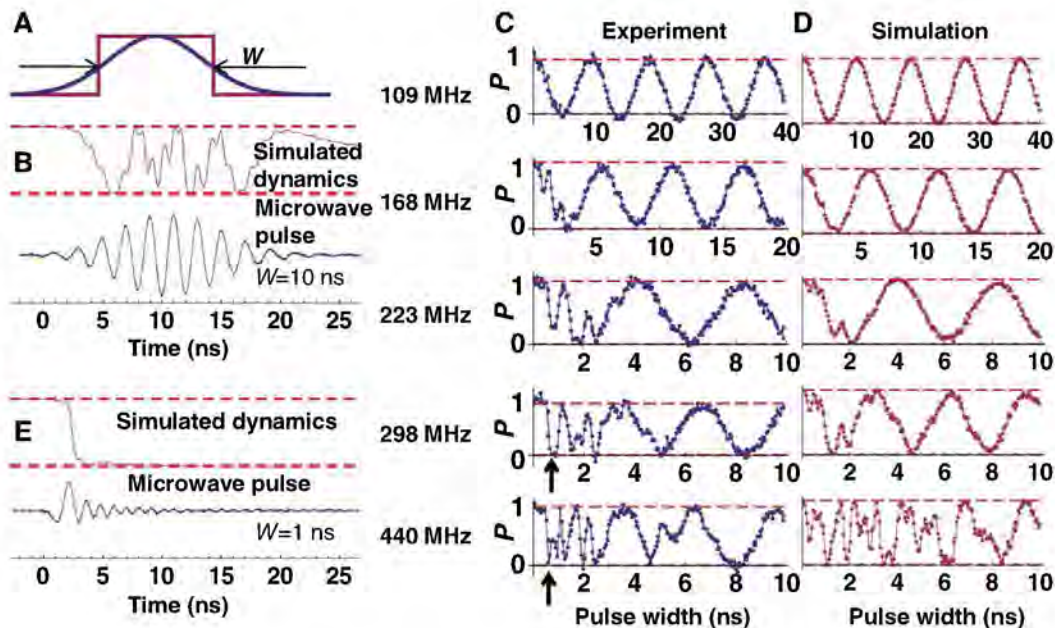


Fig. 3. (A) Comparison between the nominal envelope functions for square and Gaussian pulses. (B) Simulated time evolution of P for a $W = 10$ ns pulse with a peak $H_1 = 440$ MHz is plotted on the same scale. (C) Experimental Rabi oscillations using Gaussian pulses for five values of H_1 . Each shows I_{PL} as a function of nominal pulse width, W . The dashed red lines show the values of I_{PL} level that correspond with $P = 1$ (upper) and 0 (lower). The two black arrows indicate points where the microwave pulse produces one spin flip in roughly half a Larmor precession cycle. (D) Simulated Rabi oscillations showing the final population of $m_s = 0$ as a function of W for the same values of H_1 as in (C). (E) Simulated time evolution of P for a $W = 1$ ns pulse with a peak $H_1 = 440$ MHz is plotted on the same scale.



agreement with the experiment and the previous simulation. This correspondence shows that, although transient effects exist, the experimental data reflect the true time evolution of the spin state. In addition, some differences between the simulation and the experiment are expected because the spin dynamics in this regime are sensitive to fine details of the pulse shape (17).

As a first step toward optimizing the strong-driving field, we engineered Gaussian-shaped pulses to mitigate pulse edge effects. The pulses were normalized so that the full-width at half maximum, W , is equal to the nominal width of a square pulse with the same area (Fig. 3A). Figure 3B shows the simulated time evolution of P during a $W = 10$ ns pulse with a peak $H_1 = 440$ MHz. The simulated dynamics have anharmonic features in the center of the pulse, with both sub-nanosecond rotations and stalling, but with more gradual spin rotation accumulating in the pulse edges.

We focused on the largest driving fields in the Gaussian pulse experiment, with smaller steps in H_1 (Fig. 3C), and plotted for comparison the simulations of final population (Fig. 3D). At $H_1 = 109$ MHz, the Gaussian pulses recover sinusoidal Rabi oscillations compared with the steplike oscillations seen for the square pulses at the same driving field. As H_1 was increased, however, we saw the evolution of two separate regimes: sub-period duration pulses ($W < 2$ ns) and longer pulses. Subperiod pulses generated strongly anharmonic rotations, whereas longer pulses induced comparatively smooth Rabi oscillations with a rotation rate roughly linear in H_1 . At $H_1 = 440$ MHz, there were some features of nonlinear rotations even for large W .

An important feature of these data are the reproducible spin flips from pulses with nominally sub-nanosecond values of W (arrows, Fig. 3C). The simulations confirm that, although the pulse

has a nonresonant tail that is longer than a nanosecond because of ring-down in the amplifier, nearly all of the rotation occurs on sub-nanosecond time scales with little additional rotation from the tail. This can be seen in Fig. 3E, which shows that during the simulated evolution of P for a $W = 1$ ns pulse there is a spin flip in less than 1 ns. With additional experimental and theoretical effort, sub-Larmor cycle manipulation could be optimized for even faster quantum control of two-level systems.

As quantum information processing extends toward technology, advancements will be needed on several fronts. Understanding and controlling the dynamics in the strong-driving regime is a key step toward optimizing fast quantum control over two-level systems. When combined with rotations from Larmor precession, these results enable full quantum control at gigahertz rates. We have demonstrated that, although simple scaling of the driving field breaks down, strong driving offers opportunities to test advanced concepts of quantum control (19–21) and achieve faster state operation than expected from a driving field with conventional approaches. Moreover, by combining the millisecond coherence times in diamond (25) with the sub-nanosecond manipulation from strong-driving, more than a million coherent operations can be performed on a single NV center spin at room temperature.

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Meteorite Kr in Earth's Mantle Suggests a Late Accretionary Source for the Atmosphere

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Noble gas isotopes are key tracers of both the origin of volatiles found within planets and the processes that control their eventual distribution between planetary interiors and atmospheres. Here, we report the discovery of primordial Kr in samples derived from Earth's mantle and show it to be consistent with a meteorite or fractionated solar nebula source. The high-precision Kr and Xe isotope data together suggest that Earth's interior acquired its volatiles from accretionary material similar to average carbonaceous chondrites and that the noble gases in Earth's atmosphere and oceans are dominantly derived from later volatile capture rather than impact degassing or outgassing of the solid Earth during its main accretionary stage.

There are many possible origins for planetary gases, but there is little evidence to identify whether or not the volatiles within our planet have the same accretionary origin as

those that now form our oceans and atmosphere. After collapse of the solar nebula, planetesimals were formed from the dust within the first 0.1 to 1 million years (1). Although it took a further 100

million years of violent impacts between these planetary embryos before the terrestrial planets we see today remained, 60% of Earth's mass accumulated within the first 10 to 20 My (1, 2). Solar nebula gas may have been present for as long as 10 My after nebula collapse was initiated (3) and contemporaneous with the terrestrial planet precursors. Indeed, gravitational capture of solar nebula gases by the early Earth (4) is used as a starting point for many noble gas and volatile accretionary models of both the terrestrial atmosphere and the interior (5, 6). Alternatively, volatiles may have been carried to the early Earth by accreting dust, planetesimals (7), and cometary material (8). These sources would be composed of gases from implanted solar wind (9–11), adsorbed nebula gases (12), and minor amounts of presolar material.

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Although accretionary models of the inner planets suggest that water and other volatiles may have been acquired from cometary and other icy body material (13), others contend that impact degassing of accretionary material or early planetary degassing played a major role in atmosphere formation (14).

Within Earth's mantle, primordial $^{20}\text{Ne}/^{22}\text{Ne}$ ratios show a similarity between the terrestrial mantle value and that of solar wind that has been implanted into meteorite surfaces and the lunar regolith (9, 10). Although the source of Earth's primordial light noble gases (He and Ne) is most likely from addition of solar wind-irradiated material to the surfaces of accreting material, it is not possible to predict from this result whether heavy noble gases (Ar, Kr, and Xe) trapped within the primordial Earth have a similar implanted solar wind composition. This is because, when compared to solar elemental ratios, enrichment of Kr and Xe in chondrites by $\sim 10^4$ and 10^5 , respectively, relative to Ne require only minor input from chondritic material for it to dominate the noble gases signature (15). Identifying the original isotopic composition of the heavy noble gases within Earth is nevertheless critical in developing our understanding of the relationship and timing of volatile interaction between the deep Earth and the atmosphere (16, 17).

Magmatic CO_2 natural gases have proved to be an invaluable resource for studying mantle gases (10, 16–19). Here, we report Kr and Xe isotopic measurements of CO_2 gases to determine the isotopic composition of heavy noble gases in Earth's interior. Our samples were collected from the Bravo Dome gas field in New Mexico, United States, which produces almost pure (99%) CO_2 from the Tubb sandstone at depths of 600 to 700 m. Magmatic gas in this field has been in contact with groundwater to varying degrees, providing a spatially coherent mixture of gas with two different origins. End-member com-

positions are represented by near-pure magmatic gas in the western portions of the field and a mixture of dissolved air-derived gas and crustal radiogenic noble gases that have accumulated in the groundwater to the east (10, 16). To date, Kr and Xe measurements have been limited by the precision of single-collector instruments (16, 18, 19). To overcome these limitations, we used multi-collector noble gas mass spectrometry (20).

Isotope data of $^{86}\text{Kr}/^{82}\text{Kr}$ versus $^{84}\text{Kr}/^{82}\text{Kr}$ show a linear trend from atmospheric values to compositions with nonair ratios (Fig. 1A and table S1). Samples showing the greatest deviation from air also possess the lowest Kr concentration, as would be expected from samples with the least air contamination (fig. S1). The largest deviations from air of Kr isotopes also correlate with the highest mantle-derived $^3\text{He}/^4\text{He}$, $^{20}\text{Ne}/^{22}\text{Ne}$, and $^{124-128}\text{Xe}/^{130}\text{Xe}$ isotopic compositions observed in previous work (10, 16). Unfractionated $^{38}\text{Ar}/^{36}\text{Ar}$ data (17) and the high precision $^{124-128}\text{Xe}/^{130}\text{Xe}$ data presented here exclude mass fractionation during transport or storage as the cause of the isotopic deviations from air observed in Kr and nonradiogenic Xe. We interpret the Kr isotopic deviations from air as a resolvable primordial mantle Kr signal. Samples were corrected for ^{86}Kr and ^{84}Kr produced from U fission in the crust (Fig. 1B) to give a Kr isotopic composition for each sample that must lie on the present day mantle-air mixing line. This mixing line serves as a reference line for comparison of terrestrial data with possible primordial or accretionary volatile sources. The mixing line shown represents the minimum fission correction because correcting for fission U and Pu-derived Kr within the mantle may decrease the gradient of this mixing line further depending on the U/Pu of the mantle source (20) (fig. S4).

The air-mantle mixing line is consistent with a fractionated solar or average carbonaceous chondrite (AVCC) source for the primordial mantle Kr.

The maximum Pu/U fission correction (fig. S4) produces an air-mantle mixing line that remains within the AVCC error envelope but no longer within error of the air-fractionated solar mixing line. Importantly, our data exclude unfractionated solar nebula gas (solar) as a primordial or accretionary component now preserved in the mantle sampled by these gases. AVCC is the product of tightly bound Ar, Kr, and Xe found in carbonaceous residues of meteorites, referred to as the Q component, and other minor noble gases trapped in different phases. The Kr isotope system has the ability to distinguish between AVCC and Kr-Q. Because the air-mantle mixing line excludes Kr-Q as the sole source of Kr, the mechanism of accretion does not distinguish between the different locations of noble gases within meteorites—an important consideration for non-noble gas volatile elements.

Xe isotope data provide further perspective on the Kr observations. Samples from Bravo Dome have $^{124-136}\text{Xe}/^{130}\text{Xe}$ in excess of air values (16, 19). The light Xe isotopes are unaffected by radiogenic addition, and these deviations from air represent a resolvable primordial component in the mantle Xe record. The trend described by our $^{124}\text{Xe}/^{130}\text{Xe}$ and $^{126}\text{Xe}/^{130}\text{Xe}$ data (Fig. 2) is indistinguishable from an AVCC (or Q) component and is consistent with published well gas and mid-ocean ridge basalt (MORB) reservoir data (table S4). The Xe isotope results are also consistent with the Kr observation that the primitive heavy noble gases within Earth are sourced by a chondritic reservoir with a composition similar to that of carbonaceous chondrites or a fractionated solar nebula source. At present, solar nebula Xe cannot be resolved from AVCC without further improvement in the precision of the solar and AVCC Xe end-member values. We focus our discussion on $^{124}\text{Xe}/^{130}\text{Xe}$ and $^{126}\text{Xe}/^{130}\text{Xe}$ ratios because of possible ^{128}Xe production by neutron capture in iodine that may increase uncertainty in end-

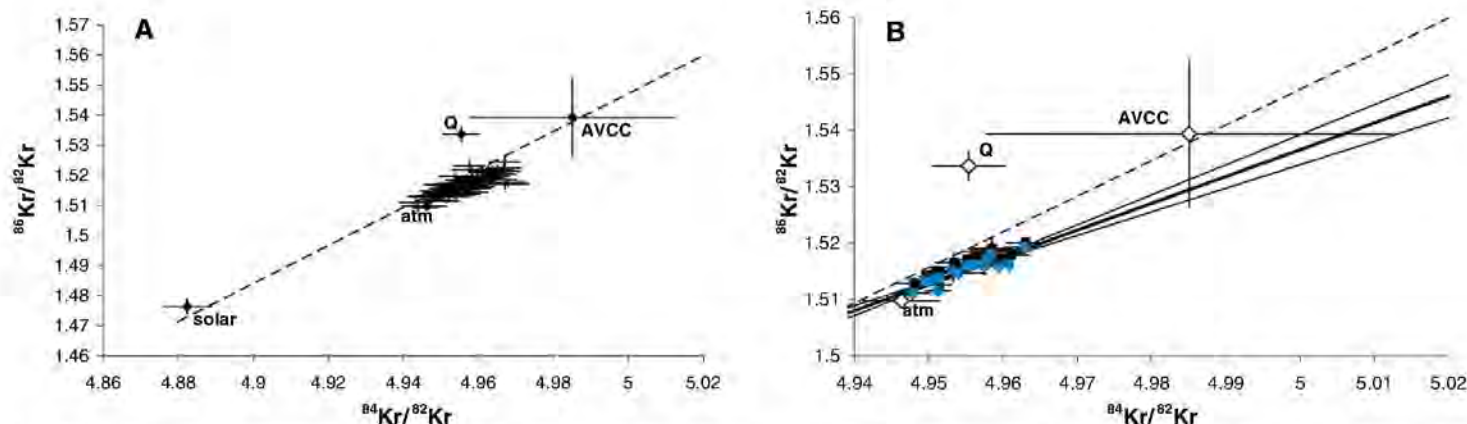


Fig. 1. Kr isotope measurements of well gases show a clear, nonsolar primordial component. (A) Individual analyses of all sample aliquots ($n = 125$) are plotted. Uncertainties in well gas data and primordial end-members are 1σ . Data are compared with those from solar-Kr, AVCC-Kr, Kr-Q, and Air-Kr (31). The dashed line represents the solar mass fractionation line. (B) Averages of each sample are plotted both as uncorrected data (black) and as crustal fission corrected (blue) (see fig. S4 for corrections involving both U and Pu fission). The bold line is a York fit (32) freely fitted through

the data. Thin black lines are 1σ error envelopes to the data. The dashed line represents the solar mass fractionation line. Fission correction is based on crustal U fission determined from ^{136}Xe excesses over air and assumes no Kr/Xe fractionation. Uncorrected data do not pass through air because of a crustal U fission component to Kr as observed previously in Xe isotopes (16); when corrected for fission, extrapolation of the best fit line passes through atmosphere. A summary of primordial end-members is presented in table S4.

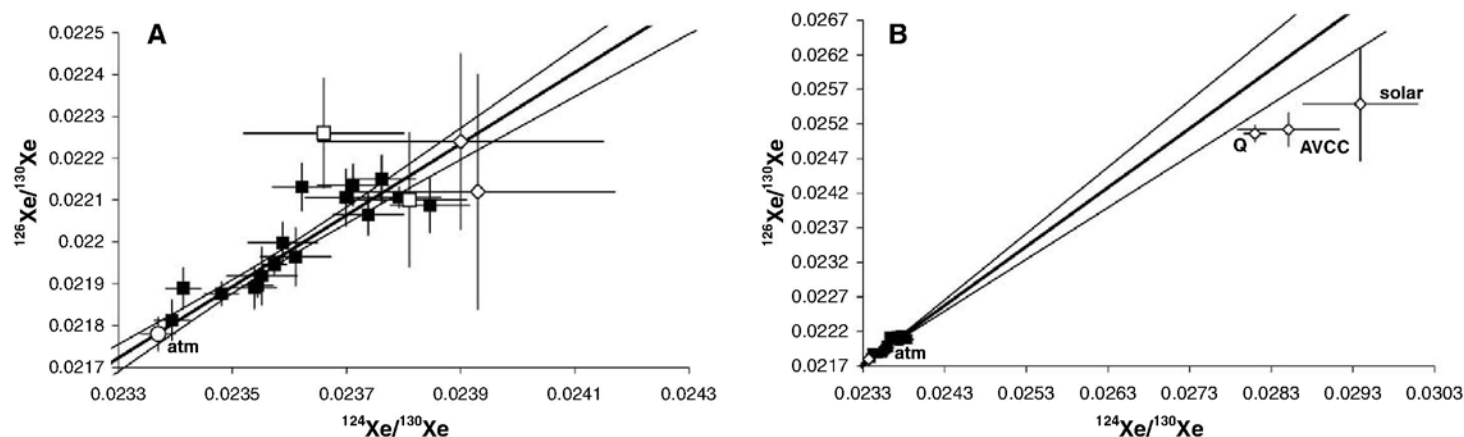


Fig. 2. Nonradiogenic Xe isotope measurements of well gases show a primordial component that is completely consistent with Kr data. **(A)** Reduced scale view to show Xe nonradiogenic isotope data, plotted as $^{126}\text{Xe}/^{130}\text{Xe}$ versus $^{124}\text{Xe}/^{130}\text{Xe}$ with errors at 1σ uncertainty. The bold line is a York fit (32), freely fitted through the well gas data presented in table S2. Thin black lines are 1σ error envelopes. Open squares are Harding County and Caroline well gases (19). Open diamonds

are MORB averages computed from (21). The open circle is modern atmosphere (atm). **(B)** Expanded scale view to incorporate possible end-members, plotted as $^{126}\text{Xe}/^{130}\text{Xe}$ versus $^{124}\text{Xe}/^{130}\text{Xe}$. The bold line is the error-weighted best fit, freely fitted through the data. Thin black lines are error envelopes of 1σ . Uncertainties in the primordial end-members are 1σ . Isotopic compositions of solar Xe, AVCC-Xe, Xe-Q, and Air-Xe are tabulated in (31) and table S4.

member $^{128}\text{Xe}/^{130}\text{Xe}$. Additional $^{124-128}\text{Xe}/^{130}\text{Xe}$ data are plotted in fig. S5.

To examine whether the well gases, which sample the subcontinental lithospheric mantle, are representative of the convecting mantle, we turn to previous observations. These show that the $^3\text{He}/^4\text{He}$, $^{20}\text{Ne}/^{22}\text{Ne}$, $^{40}\text{Ar}/^{36}\text{Ar}$, $^{124,126,128,129}\text{Xe}/^{130}\text{Xe}$, and $^3\text{He}/^{20}\text{Ne}/^{36}\text{Ar}/^{84}\text{Kr}/^{130}\text{Xe}$ of the well gas mantle source is almost indistinguishable from that of MORB, represented by the 2PID43 popping rock (10, 16, 21, 22). In addition, both reservoirs have an identical $^{36}\text{Ar}/^{84}\text{Kr}/^{130}\text{Xe}$ abundance composition that is indistinguishable from seawater and a small (10%) sedimentary contribution, itself unique in isotope character and composition in the solar system. This is interpreted as evidence for seawater recycling into both mantle reservoirs and argues against isolation of these reservoirs from each other over geological time (16). The only significant difference between the MORB and well gas mantle reservoirs is in their respective Pu-derived Xe content, which has been interpreted as reflecting a different closure age and isolation of the two reservoirs for over 4 Gy (21). Many mantle models balance an accretionary gas-rich reservoir flux into the convecting mantle with radiogenic in-growth and a mantle degassing with possible atmosphere recycling to achieve the present-day convecting mantle noble gas abundance and isotopic composition [e.g., variants on (23)]. In particular, we argue that there is no a priori reason why the nonradiogenic primordial Kr and Xe components should differ between reservoirs when both mantle reservoirs preserve indistinguishable nonradiogenic primordial He, Ne, and Xe. The overwhelming similarities between the reservoirs lead us to conclude that the extension of the mantle sampled by the well gases to the rest of the mantle is indeed valid.

Taken together, the Kr and Xe data are consistent with the early mantle acquiring a composition that is mass-fractionated and depleted in

the lighter isotopes relative to the solar nebula (Fig. 1). A source that provides both this signature and a mechanism of delivery to the early Earth is accretion of meteoritic material similar to AVCC (Figs. 1 and 2). A similar explanation has been argued to preserve and deliver a solar wind-implanted Ne isotope signature to Earth's early mantle (9, 10). We nevertheless cannot preclude the possibility of other more-complex processes that mass-fractionate solar nebula gases and introduce these into the early Earth's interior.

Planetary evolution models often argue that the unique noble gas elemental and isotopic character of the terrestrial atmosphere can be explained by a combination of fractionation in the atmosphere and deep planetary outgassing of noble gases with isotopic compositions similar to the solar nebula (5, 24, 25). These models are attractive because they invoke preferential loss of the light elements and isotopes during hydrodynamic escape (i.e., mass-fractionating gas loss induced by upward drag from escaping hydrogen). The energy for this process may originate from either impacts or enhanced ultraviolet radiation from the early Sun. This light isotope depletion is broadly seen in Earth's atmosphere relative to possible solar system reservoirs. The heavy noble gases in Earth's atmosphere are assumed to have evolved together through mass fractionation during early atmosphere loss (5). In such scenarios, extensive fractionation of Xe isotopes required to reduce $^{124-128}\text{Xe}/^{130}\text{Xe}$ ratios from solarlike values to those observed in the atmosphere would also have produced fractionation in Kr isotopes to a much greater extent than that observed in the atmosphere today. This type of model requires subsequent input of solar Kr, but negligible additional solar Xe, to buffer these ratios back to modern-day air (5). Earth's mantle has been assumed to provide the buffering source (5).

Our results, however, show that Earth's mantle does not presently contain significant unfraction-

ated solar nebula Kr. If a solar nebula Kr component was ever present in the early Earth's mantle, evidence for it has been erased by degassing and overprinting with Kr that is isotopically heavier than air (Fig. 1). This "heavy" Kr cannot generate modern atmosphere through degassing followed by mass fractionation during atmosphere loss. We are therefore led to the conclusion that neither early outgassing nor impact-driven degassing of the early mantle represented by these gases are responsible for generating the noble gases in the present-day atmosphere. The absence of resolvable solar nebula Kr in the mantle precludes any role of this source in the buffering of heavily fractionated Kr (necessitated by Xe fractionation) back to atmospheric Kr.

We suggest that, although the Kr and Xe trapped in the mantle are primary signatures of the accreting Earth, they play only a minor role in determining the noble gas composition of the modern atmosphere. It is possible that a fractionated solar atmosphere may have been preserved as a remnant of major impact events, perhaps as late as 60 My after the start of the solar system (26), if atmosphere loss during Moon formation is substantial (27, 28). However, to achieve the approximately solar Kr/Xe ratios and isotopic compositions in the present atmosphere, an additional high Kr/Xe source is still required. Experiments on amorphous water ice suggest cometary ices could provide such a reservoir (29, 30). Earth's modern atmosphere is then a mixture of a relatively late fractionated solar gas and an even later cometary source. A similar conceptual model has been proposed as an explanation for why Xe is unexpectedly depleted in Earth's atmosphere relative to Kr (30).

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Materials and Methods

Figs. S1 to S5

Tables S1 to S4

References

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Evolution of Organic Aerosols in the Atmosphere

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Organic aerosol (OA) particles affect climate forcing and human health, but their sources and evolution remain poorly characterized. We present a unifying model framework describing the atmospheric evolution of OA that is constrained by high-time-resolution measurements of its composition, volatility, and oxidation state. OA and OA precursor gases evolve by becoming increasingly oxidized, less volatile, and more hygroscopic, leading to the formation of oxygenated organic aerosol (OOA), with concentrations comparable to those of sulfate aerosol throughout the Northern Hemisphere. Our model framework captures the dynamic aging behavior observed in both the atmosphere and laboratory: It can serve as a basis for improving parameterizations in regional and global models.

Submicron atmospheric aerosols exert a highly uncertain effect on radiative climate forcing (1) and have serious impacts on human health (2). Organic aerosol (OA) makes up a large fraction (20 to 90%) of the submicron particulate mass (3, 4). However, OA sources, atmospheric processing, and removal are very uncertain. Primary OA (POA) is directly emitted from fossil fuel combustion, biomass burning, and other sources, but the atmospheric evolution of POA after emission remains poorly characterized (3, 4). Recent results show that secondary OA (SOA), formed by atmospheric oxidation of gas-phase species, accounts for a large fraction of the OA burden (3, 5–9). Despite much recent progress in our understanding of SOA formation chemistry (10), current “bottom-up” models based on parameterizations of laboratory experiments cannot explain the magnitude and evolution of atmospheric SOA (5–9). Explicit chemical models are still not able to predict ambient SOA con-

centrations or degree of oxidation accurately, and they are too complex for large-scale models (11). A better understanding of the chemical evolution of OA is required to reduce unacceptable aerosol-related uncertainties in global climate simulations (12) and to improve air quality (13).

Here we integrate observations and modeling to better characterize the physical and chemical properties and climate effects of OA. Field and laboratory data show that the volatility and oxidation state of organics can be used to build a two-dimensional (2D) modeling framework that maps the evolution of atmospheric OA. The measurements and model reveal OA to be a highly dynamic system, tightly coupled to gas-phase oxidation chemistry. Gas-phase reactions transform OA constituents, and the OA itself is an intermediate, often forming from gas-phase precursors and ultimately returning, in part, to gas-phase products. The framework, though computationally inexpensive, allows an accurate representation of OA

in regional and global climate and air-quality models used for policy assessments.

The aerosol mass spectrometer (AMS) detects OA quantitatively by combining thermal vaporization and electron ionization (EI) (14). Factor analysis of AMS data (FA-AMS) (3, 15–17) demonstrates that AMS data contain sufficient information to differentiate several types of OA and to determine their dry oxygen content (18). FA-AMS is based on the total OA mass and avoids the challenges of techniques based on molecular tracers with highly variable tracer:OA ratios (19) that may not be stable against atmospheric oxidation (20). Figure 1 summarizes FA-AMS results at many locations in the Northern Hemisphere, with typical high-resolution component spectra shown in fig. S1 (21). POA from fossil fuel combustion and other urban sources [hydrocarbon-like OA (HOA)] and biomass-burning OA (BBOA) have been identified in multiple studies. However, most OA mass at many locations is oxygenated organic aerosol (OOA) (3), characterized by its high oxygen content, with an atomic O:C ratio (an indicator of oxidation state) of 0.25 to ~1 for highly aged OA (18). There is strong evidence that most atmospheric OOA is secondary: Increases in OOA are strongly correlated with photochemical activity (7, 22) and other secondary species (7, 16, 17, 22), and OOA levels are consistent with SOA estimates using other methods (13, 15).

At many locations, FA-AMS identifies two subtypes of OOA that differ in volatility and O:C (Fig. 1). Volatility and O:C are generally inversely correlated (16–18, 23, 24). Low-volatility OOA (LV-OOA, empirical formula $\sim C_8O_{5.5}H_{10}$) is strongly correlated with nonvolatile secondary species such as sulfate and has a high O:C (Fig. 1, inset), consistent with regional, heavily aged OA. Semi-volatile OOA (SV-OOA, empirical formula $\sim C_8O_3H_{11}$) has a higher correlation with semivolatile species such as ammonium nitrate and ammonium chloride and has a lower O:C, consistent with less-photochemically aged OA. These two OOA subtypes offer a lumped description of SOA components based on their distinct physicochemical properties. The relative concentrations of the OOA subtypes depend on both ambient temperature and photochemistry. For the three sites with both win-

ter and summer measurements, for example, SV-OOA was observed only during the summer, when the dynamic range in ambient temperature and photochemical conditions is larger.

Recent field and laboratory experiments illustrate the fact that atmospheric oxidation reactions result in the dynamic evolution of OA properties with age. This evolution contrasts sharply with the

relatively static nature of sulfate aerosol. In general, atmospheric SV-OOA corresponds to fresh SOA that evolves into LV-OOA with additional photochemical processing. Figure 2, A and B, present data acquired around Mexico City aboard the National Center for Atmospheric Research/National Science Foundation C-130 aircraft during the Megacity Initiative: Local and Global Research Observations (MILAGRO) campaign (25). This megacity experiences substantial particulate pollution, including intense SOA formation (7, 18, 22, 23, 25). The aircraft flew over a ground supersite located inside the city (designated as T0) and two sites 30 and 63 km downwind (designated T1 and T2) in the afternoon, corresponding to approximate transport times of 0, 3, and 6 hours from the urban area. In the urban area (T0), SV-OOA was already dominant, consistent with previous observations (7), but the fraction of OOA, O:C, and the relative LV-OOA contribution all increased with aging (T0→T1→T2).

A similar transformation has also been observed in the laboratory (Fig. 2, C to F) for various types of OA (21). SOA formed from the oxidation of α -pinene becomes more similar to ambient SV-OOA after some aging and then evolves with continued oxidation to become increasingly similar to ambient LV-OOA (Fig. 2C). SOA formation and heterogeneous oxidation from primary diesel emissions, biomass-burning smoke, and the POA surrogate squalane result in strikingly similar transformations (Fig. 2, D to F). The bulk OA spectra in each experiment initially resemble the appropriate source aerosol spectra, but as photochemistry proceeds, their signature is transformed and the laboratory OA spectra become more similar first to that of ambient SV-OOA and then increasingly to that of LV-OOA. These observations, when taken together, indicate that atmospheric oxidation of OA converges toward highly aged LV-OOA regardless of the original OA source, with the original source signature being replaced by that of atmospheric oxidation. This is consistent with the previously reported ubiquity in atmospheric OA of humic-like substances (HULIS), which are complex mixtures of high-molecular-weight polycarboxylic acids that are similar to fulvic acids in soil organic matter (26).

An important property of aerosols is hygroscopicity (propensity to absorb water vapor). A more hygroscopic particle of a given size will grow more under humid conditions, scattering more incident light; it will also be more likely to form cloud droplets. Both phenomena strongly influence the radiative forcing of climate through the direct and indirect effects of aerosols (1). The dependence of hygroscopicity on particle composition can be represented with the single parameter κ (27). Figure 3 shows the relationship between organic O:C and κ for ambient aerosols in urban, forested, and remote locations and also for SOA formed in laboratory chambers from three different precursors (21); O:C and κ were determined by AMS and hygroscopicity tandem differential mobility analyzer (HTDMA) (28) measurements, respectively. A trend of increas-

ing hygroscopicity with increasing O:C is robust. This strongly suggests that a model must reproduce the evolution of OA shown in Fig. 2 to estimate the variations in OA hygroscopicity, allowing the effects of OA on global climate to be determined more accurately in atmospheric models.

Traditional SOA models are based on the parameterization of smog-chamber experiments, often using a two-product absorptive partitioning scheme (10). These models typically do not capture either the amount of SOA or the substantial aging observed in field experiments described above (7). Recently, Robinson *et al.* (29) proposed an OA model scheme based on lumping species into volatility bins of a basis set (specified as decades in saturation concentration, C^* , at 298 K). This resulted in improved agreement between regional model predictions and ambient measurements. However, simplified lumping schemes based only on volatility cannot represent the broad diversity in physicochemical properties of organic species, such as polarity, solubility, carbon number, and reactivity, and thus may not reproduce the formation rates, properties, or atmospheric fates of OA.

The discussion above underscores the fact that the oxygen content and volatility of OA evolve with photochemical processing. This motivated development of a 2D volatility basis set (2D-VBS) modeling framework using OA volatility (C^*) and oxidation state (here approximated by oxygen content, O:C) as its two basis vectors. Because these two OA properties can be measured in near-real time, this framework can be constrained and directly verified with experimental data, which is an advantage over a previously proposed basis set based on carbon number and polarity (30). Moreover, this framework could be used to estimate OA hygroscopicity and would thus introduce an important simplification in atmospheric models.

As shown in Fig. 4, the 2D-VBS (21) lumps species with $C^* < 10^7 \mu\text{g m}^{-3}$ into bins that are spaced evenly in C^* and O:C space. Each bin includes many organic compounds, spanning only a narrow range of carbon numbers. All constituents are assumed to form a quasi-ideal solution according to standard partitioning theory (31). In the atmosphere, only species with $C^* < 10 \mu\text{g m}^{-3}$ typically partition substantially into the aerosol (8).

Figure 4 shows the location of the OOA factors in the 2D-VBS. Most ambient OA is a mixture of LV-OOA and SV-OOA, with $0.25 < \text{O:C} < 1$ and low C^* . Most primary emissions lie along or near the x axis (low O:C, various C^*). Photochemical reactions cause material to evolve in the 2D space. A key question is, how do primary gas and particle emissions age to become LV-OOA?

The 2D-VBS simulates photochemical aging using a functionalization kernel and a fragmentation kernel, a branching ratio between these two pathways, and a simple representation of differing homogeneous and heterogeneous oxidation by OH (Fig. 4C) (21). In the current implementation, the first generation of oxidation is modeled with explicit chemistry but the later generations of oxidation are phenomenological, with param-

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eters consistent with our current understanding of atmospheric chemistry. It can be applied to material produced from any precursor.

To test whether the model can reproduce the transformations of atmospheric OA shown in Fig. 2, the simulated formation and aging chemistry of α -pinene SOA (and the attendant vapors) are shown in Fig. 4. The full (vapor and particle) first-generation distribution from the α -pinene + O_3 reaction (derived from chamber data) is shown with blue contours in Fig. 4A, having $1 < C^* < 10^7 \mu\text{g m}^{-3}$ and $0.1 < O:C < 0.4$. Lower-volatility products to the upper left of the blue contours condense to form SOA. The reaction of the first-generation particle and vapor distribution with OH is modeled with functionalization reactions that generate products that are roughly within the limits indicated by the red dashed lines in Fig. 4A. The predicted condensed-phase products after 1.5 lifetimes of OH oxidation are shown with purple contours in Fig. 4A and the yellow star in Fig. 4, A and B. The model predicts a tripling

of SOA mass by the end of the second generation of oxidation. This increase is also accompanied by an increase in O:C, shown in Fig. 4B, and a drop in average C^* of the aerosol. The predictions in Fig. 4 are consistent with observations of *cis*-pinonic acid, a typical first-generation reaction product, and of its OH oxidation product α,α -dimethyltricarballic acid (8). Moreover, the simulation results reproduce the SV-OOA to LV-OOA transformation observed in the laboratory experiments on α -pinene SOA aging (Fig. 2C).

The model predicts very similar outcomes for the aging of other SOA precursors, including the evaporated diesel and biomass-burning smoke shown in Fig. 2, E and F. Most of the aging in these simulations occurs via gas-phase oxidation of semivolatile vapors. OOA formation occurs mainly via condensation of the less volatile products of these aging reactions on accumulation-mode particles, where OOA principally resides (15). However, in all of these cases, the majority of the oxidation products in the model are higher-volatility gases.

Although the current implementation of the framework considers aging only by reactions with OH, other aging mechanisms, such as oligomerization or the addition of hydrated glyoxal to a semivolatile organic in the condensed phase (32), could be incorporated into the framework. These mechanisms represent other means of substantially increasing O:C while reducing C^* by several decades.

OA is dynamic and continually evolves in the atmosphere; this evolution strongly influences the effects of particulate matter on climate and air quality. The complex evolution of OA contrasts with the simpler behavior of sulfate, which is irreversibly oxidized and condensed. Current modeling frameworks for OA are constructed in an analogous way to those for sulfate, with either no aging or one-step oxidation. Here we have presented a unifying framework describing the atmospheric evolution of OA, which is directly connected to worldwide observations and experimentally verifiable and can be used to evaluate and form the basis of practical phenomenological modeling

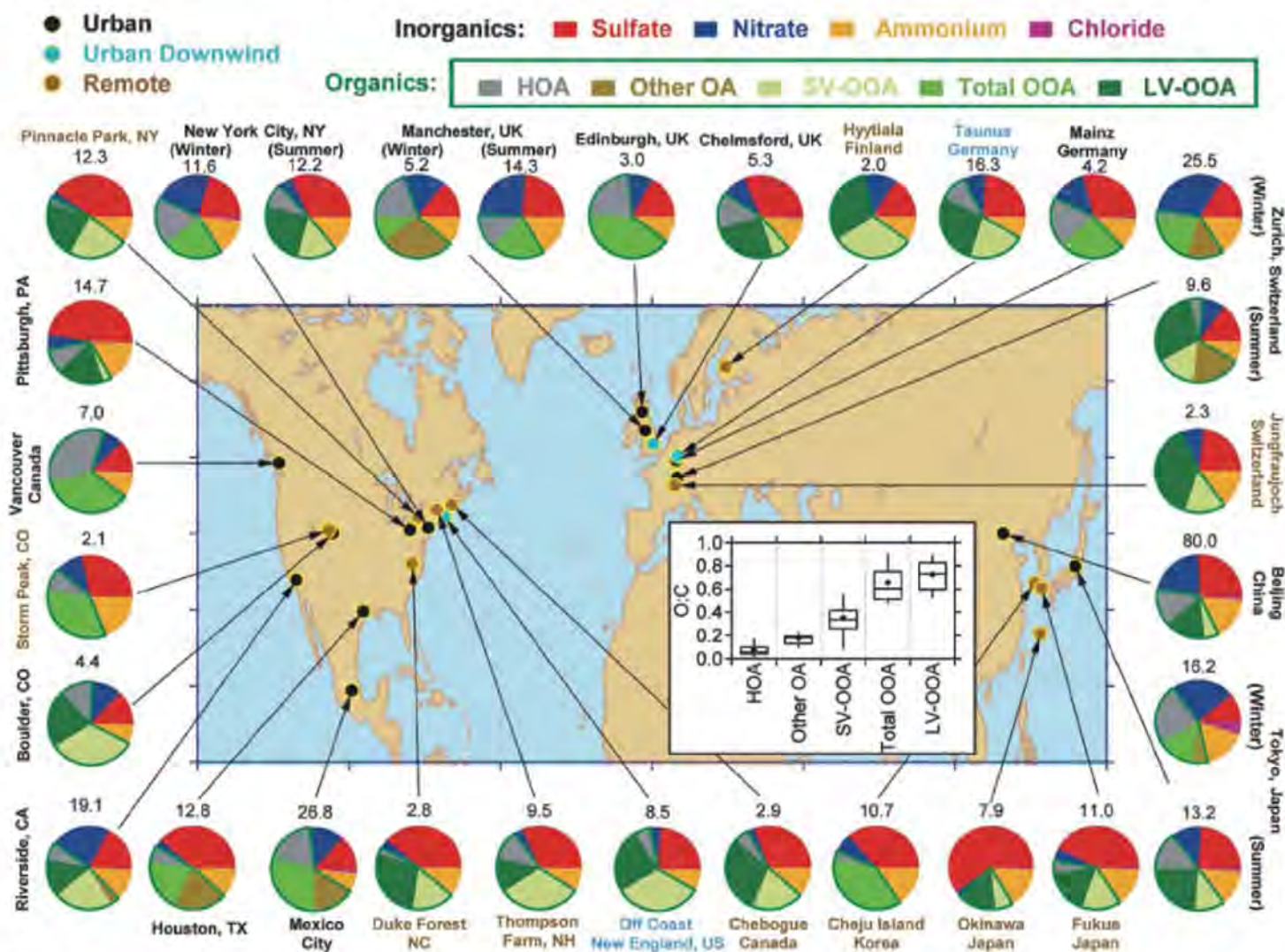


Fig. 1. Total mass concentration (in micrograms per cubic meter) and mass fractions of nonrefractory inorganic species and organic components in sub-micrometer aerosols measured with the AMS at multiple surface locations in the Northern Hemisphere (21). The organic components were obtained with FA-AMS methods (3, 15–17). In some studies, the FA-AMS methods identified

one OOA factor, whereas in other locations, two types, SV-OOA and LV-OOA, were identified. HOA is a surrogate for urban primary OA, and Other OA includes primary OAs other than HOA that have been identified in several studies, including BBOA. (Inset) Distributions of O:C for the OA components identified at the different sites, calculated according to (18).

Fig. 2. Field and laboratory data of OA evolution with photochemical aging. (**A** and **B**) Atmospheric aging of OA above the T0→T1→T2 sites in and around Mexico City (corresponding to approximate transport times of 0, 3, and 6 hours from the urban area) as measured from the C-130 aircraft during the MILAGRO field experiment. OA/ Δ CO, where Δ CO is the measured CO minus a Northern Hemispheric background of 100 parts per billion by volume, is plotted in (**B**) to correct for dilution of the air mass. Biomass burning was suppressed by rain during this period. (**C** to **F**) Evolution of OA composition during photochemical aging in laboratory reaction chambers of (**C**) α -pinene SOA, (**D**) squalane (a liquid hydrocarbon used as a surrogate for reduced primary OA), (**E**) diesel exhaust, and (**F**) biomass-burning smoke. In (**C**) to (**F**), the increased degree of oxidation and similarity to ambient OOA spectra are indicated by the Pearson correlation coefficients (R^2) between the evolving total OA spectra in each experiment and the SV-OOA and LV-OOA spectra derived from the Mexico City field data set. The similarity to the initial source spectra decreases in all cases: For α -pinene and squalane, the evolving OA is compared to the original OA, whereas for diesel exhaust and wood smoke, it is compared with ambient HOA and BBOA from Mexico City. Dashed lines are included to guide the eye.

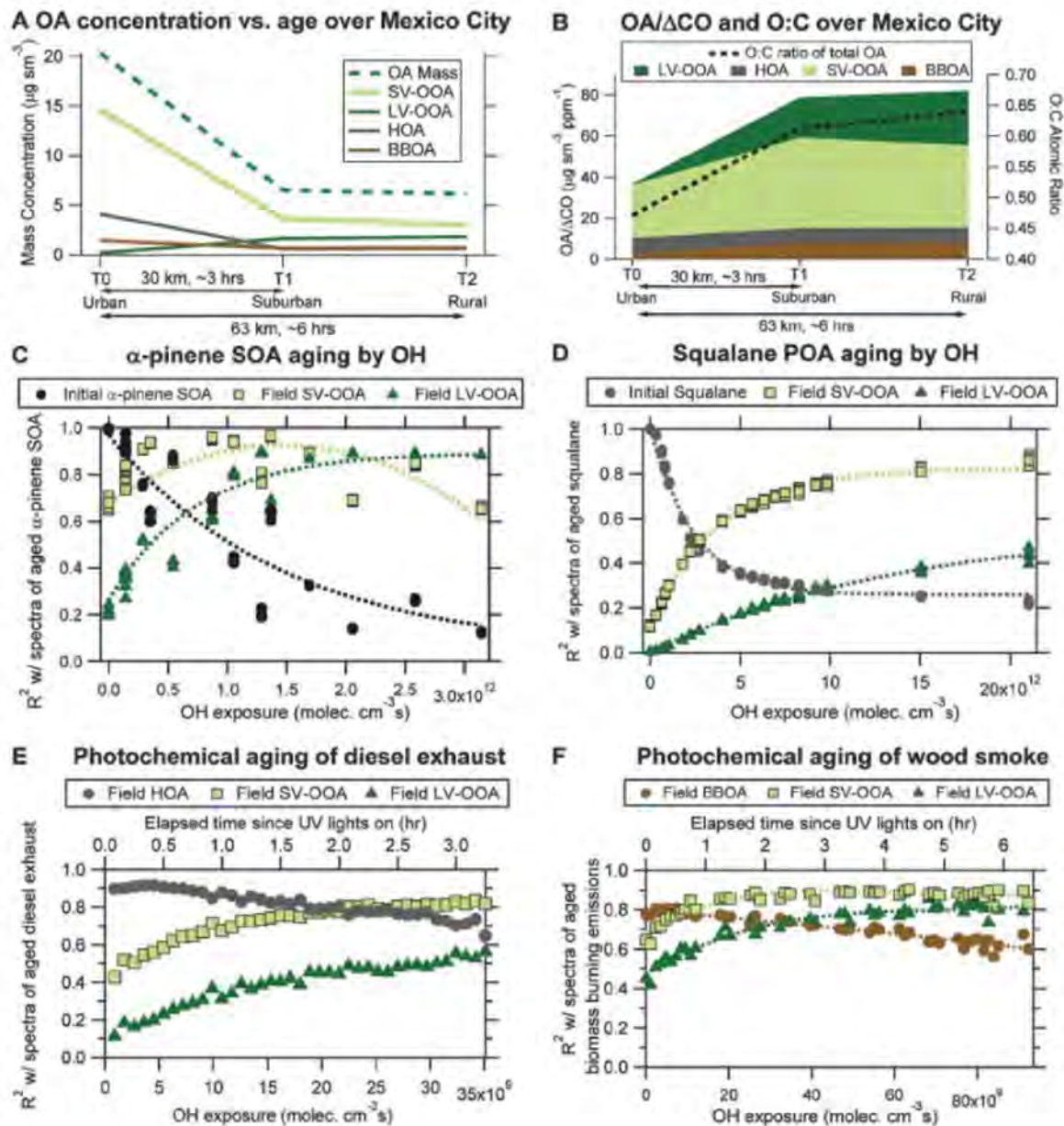


Fig. 3. Relationship between O:C and hygroscopicity (κ , or equivalently the particle growth factor at 95% relative humidity) of OA for several field data sets (a high-altitude site at Jungfraujoch, Switzerland; above Mexico City, a polluted megacity; and at the forested site of Hyytiälä, Finland) and for laboratory smog chamber SOA (21). TMB, trimethylbenzene. Error bars represent the uncertainties in O:C and κ_{org} (org, organic) and are shown for only a few data points to reduce visual clutter. G_F , growth factor; a_w , water activity.

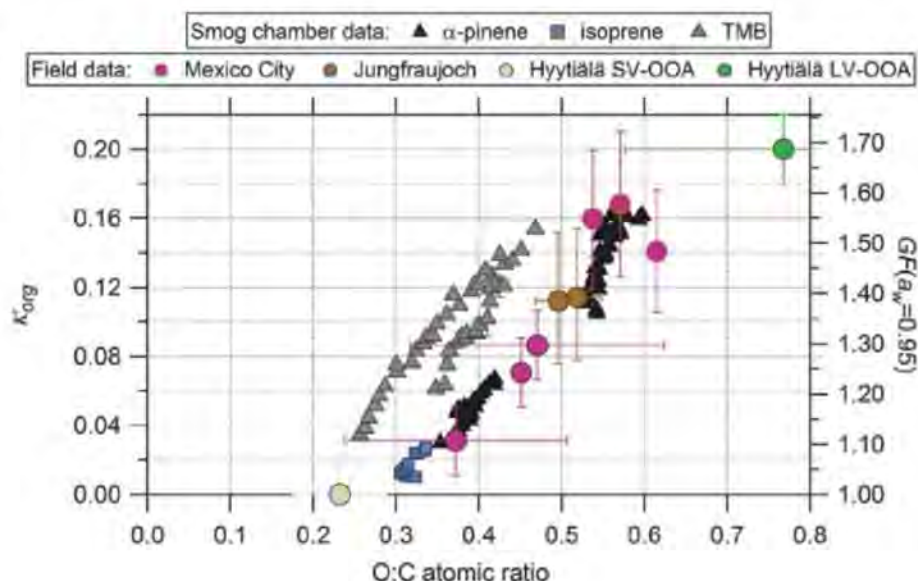
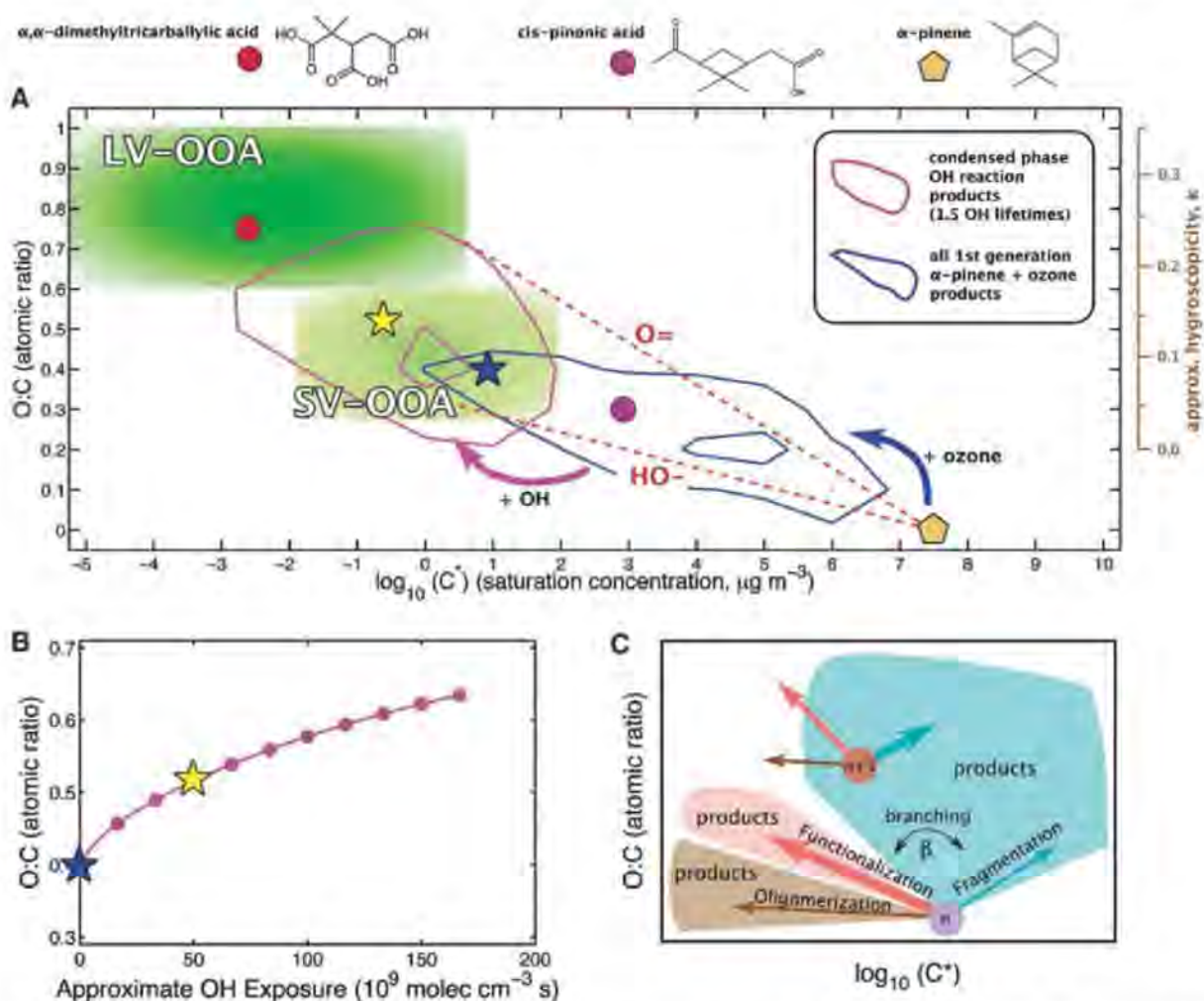


Fig. 4. (A) 2D framework for OA aging. The x axis is volatility ($\log_{10}$ of C^* at 298 K). The y axis is oxidation state, approximated by O:C. The secondary y axis shows the approximate κ of α -pinene SOA from Fig. 3. Compounds with $C^* \leq C_{OA}$ (the organic aerosol concentration, typically 1 to $10 \mu\text{g m}^{-3}$) favor the condensed phase. Those with $C^* > C_{OA}$ favor the gas phase. The OOA factors described in Figs. 1 to 3 fall in this 2D space as shown by the green areas, with LV-OOA being less volatile and more oxidized than SV-OOA. We modeled the initial oxidation of common precursors with explicit chemistry, but later-generation oxidation applies to material produced from any precursor. α -pinene (brown pentagon) is an example. All products from the α -pinene + ozone reaction, modeled explicitly, are distributed according to the blue contours; the material at low C^* and high O:C forms SOA (with mean properties indicated by the blue star). Typical effects of adding (=O) and (–OH) functionality to a C_{10} backbone are shown with red dashed lines, and a common first-generation product, *cis*-pinonic acid, is shown with a magenta dot. After forming α -pinene SOA explicitly, we modeled subsequent aging reactions with OH within the 2D-VBS. A representative second-generation product, a C_8 triacid, is shown with a crimson dot within the LV-OOA range. Modeled condensed-phase products after 1.5 lifetimes of OH oxidation are shown with purple contours. The mass-weighted average is indicated by the yellow star. This simulation reproduces a substantial shift toward ambient OOA characteristics, indicated by the shift between the blue and yellow stars. (B)



Evolution of condensed-phase O:C versus approximate OH exposure for simulated aging (similar to Fig. 2C). The blue and yellow stars for organic aerosol in (A) are shown. (C) Oxidation can occur in the gas or condensed phase, and reactions transform material as shown (21). Reactions form three categories: fragmentation, functionalization, or oligomerization, based on whether the carbon number decreases, stays the same, or increases. Here we model the first two pathways. The branching ratio (β) between these pathways is critical. Functionalization will reduce volatility considerably, whereas fragmentation can generate more-volatile species, which are less likely to partition to the OA.

approaches. The combination of measurements and the modeling framework implies that most OA is an intermediate state of organic material, between primary emissions of reduced species and highly oxidized volatile products (CO and CO_2). Future models, inventories, and measurements will almost certainly need to account for the dynamic sources and sinks of OA to accurately predict regional and global OA distributions and properties and thus the associated health and climate effects.

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A Complete Skeleton of a Late Triassic Saurischian and the Early Evolution of Dinosaurs

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Characterizing the evolutionary history of early dinosaurs is central to understanding their rise and diversification in the Late Triassic. However, fossils from basal lineages are rare. A new theropod dinosaur from New Mexico is a representative of the early North American diversification. Known from several nearly complete skeletons, it reveals a mosaic of plesiomorphic and derived features that clarify early saurischian dinosaur evolution and provide evidence for the antiquity of novel avian character systems including skeletal pneumaticity. The taxon further reveals latitudinal differences among saurischian assemblages during the Late Triassic, demonstrates that the theropod fauna from the Late Triassic of North America was not endemic, and suggests that intercontinental dispersal was prevalent during this time.

By the Late Triassic (~230 million years ago), Dinosauria had diversified into three major lineages: Sauropodomorpha, Theropoda, and Ornithischia (1, 2). In comparison to the later Mesozoic, fossils of Triassic early dinosaurs and their closest relatives are generally rare, fragmentary, and incomplete (3, 4). Indeed, the record from the Ischigualasto Formation, which provides some of the most detailed information on early dinosaur evolution (1, 2, 5), reveals that dinosaur specimens constitute less than 6% of the tetrapod assemblage (6). This depauperate fossil record has limited our understanding of early dinosaur interrelationships, diversification, and paleobiogeography, and the origin of modern avian morphologies during a critical interval of Mesozoic climate change and faunal turnover (7–9).

Here we report on a new carnivorous dinosaur represented by two nearly complete skeletons and several other partial specimens collected in a tightly associated small grouping at a single locality. Characterization of this taxon's morphology and phylogenetic history enables us to solidify basal saurischian dinosaur relationships and bears directly on the evolution of early saurischian character systems, paleobiogeography, and diversification.

Systematic paleontology: Archosauria Cope 1869 *sensu* Gauthier and Padian 1985. Dinosauria

Owen 1842 *sensu* Padian and May 1993. Theropoda Marsh 1881 *sensu* Gauthier 1986. *Tawa hallae*, nov. taxa.

Etymology. *Tawa*, Hopi name for the Puebloan sun god; *hallae*, after Ruth Hall, who collected many of the specimens that formed the genesis of the Ghost Ranch Ruth Hall Museum of Paleontology (GR) collections. **Holotype.** GR 241. A nearly complete associated but disarticulated skull and postcranial skeleton. **Paratypes.** A nearly complete skeleton of a larger individual (GR 242) and at least six other individuals found in the same area of the quarry [see supporting

online material (SOM) (10)] including femora, pelvis, and tail (GR 155) and cervical vertebrae (GR 243). A complete right femur (GR 244) is from Hayden Quarry (HQ) site 3. **Locality and horizon.** Site 2, HQ, Ghost Ranch, Rio Arriba County, New Mexico, USA. The HQ has been dated to ~215 to 213 million years ago (11) and is in the lower portion of the Petrified Forest Member of the Upper Triassic Chinle Formation (12).

Diagnosis. A theropod diagnosed by the following combination of characters (autapomorphies are noted by an asterisk here and in Figs. 1 and 2): Prootics meet on the ventral midline of the endocranial cavity; anterior tympanic recess greatly enlarged on the anterior surface of the basioccipital and extending onto prootic and parabasisphenoid; deep recess on the postero-dorsal base of paroccipital process*; sharp ridge extending dorsoventrally on the middle of the posterior face of the basal tuber*; incomplete ligamental sulcus on the posterior side of the femoral head and semicircular muscle scar/excavation on the posterior face of the femoral head*; small semicircular excavation on the posterior margin of the medial posterior condyle of the proximal end of the tibia*; "step" on ventral surface of the astragalus*; and metatarsal I similar in length to other metatarsals. See SOM for differential diagnosis (10).

Description. The holotype material is a juvenile or subadult individual, based on comparison to the largest femur among the referred material and the open braincase and neurocentral sutures. The premaxilla (Fig. 1) is similar to that

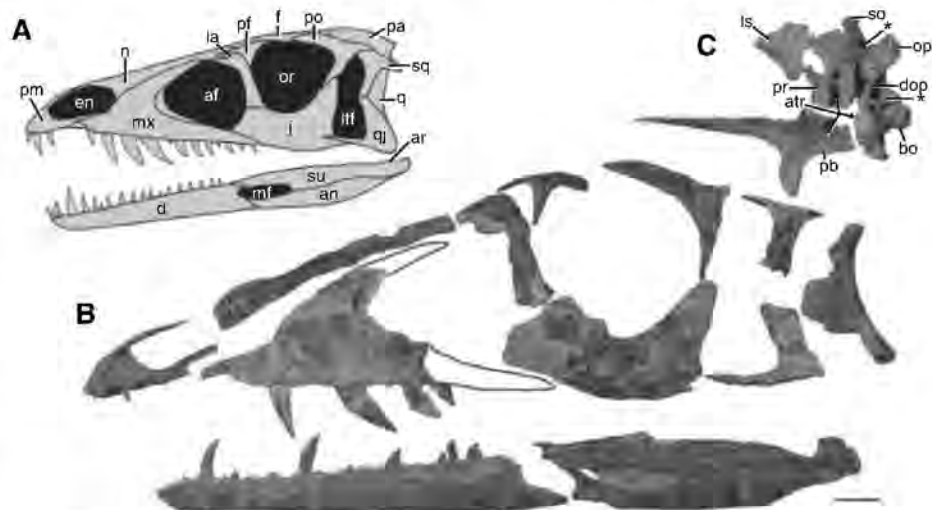


Fig. 1. The skull of *T. hallae* nov. taxa. (A) Reconstruction of the skull in lateral view. (B) The preserved skull elements of the holotype of *T. hallae* (GR 241) in left lateral view (j, qj, and the posterior portion of the mandible were reversed, and the matrix was digitally erased from the pm, mx, and n. The processes of the maxilla are present but obscured by matrix in lateral view, so they are represented here in outline). (C) Braincase of *T. hallae* in left lateral view (parabasisphenoid reversed). Abbreviations in the figure are as follows: antorbital fenestra (af), angular (an), articular (ar), anterior tympanic recess (atr), basioccipital (bo), dentary (d), descending process of the opisthotic (dop), external naris (en), frontal (f), jugal (j), lacrimal (la), laterosphenoid (ls), lower temporal fenestra (ltf), maxilla (mx), mandibular fenestra (mf), nasal (n), opisthotic (op), orbit (or), parabasisphenoid (pb), prefrontal (pf), premaxilla (pm), postorbital (po), prootic (pr), quadrate (q), quadratojugal (qj), supraoccipital (so), squamosal (sq), and surangular (su). Scale bar, 1 cm. Autapomorphies are noted by an asterisk.

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of coelophysids in possessing unserrated premaxillary teeth and a narial process of the premaxilla that is elongate and forms a low angle with the alveolar margin. It differs from neotheropods by having a relatively tall maxillary process that extends dorsally beyond the posterior border of the naris as in *Herrerasaurus*. In dorsal view, the premaxilla-nasal suture is simple, lacking the W-shaped morphology present in Neotheropoda. Like basal neotheropods (such as *Coelophysis bauri* and *Dilophosaurus wetherilli*), a subnarial gap is expressed between the premaxilla and maxilla, but unlike these taxa, *Tawa* and *Herrerasaurus* lack an extensive antorbital fossa on the lateral surface of the maxilla. A concave narial fossa is located on the anterolateral surface of the nasal. A lateral ridge on the nasal forms the dorsal border to the antorbital fossa, similar to *Eoraptor lunensis* and *C. bauri*. Unlike most basal neotheropods, in *Tawa* the jugal participates in the antorbital fenestra and lacks distinct lateral ridges on the maxilla and jugal. The lacrimal is anterodorsally inclined, as in *Herrerasaurus* and more basal dinosaurs. The anterior process of the quadratojugal is elongate as in *C. bauri* but unlike that in *Herrerasaurus*.

Tawa lacks several braincase character states present in most other dinosauriforms. Absent character states include a reduced and medially

recessed descending process of the opisthotic [the crista interfenestralis (10)] and a metotic strut. A weak parabasisphenoid recess is present on the ventral surface of the braincase, similar to that in *Herrerasaurus*, *Eoraptor*, and Neotheropoda.

The vertebral column (Fig. 2) shares several apomorphic features with neotheropods. Cervical vertebrae preserve anterior pneumatic pleurocoels (as rimmed fossae) and anterior and posterior infrazygapophyseal fossae; these features are present in all basal neotheropods. The diapophyses and parapophyses of the anterior to midcervical vertebrae are close together and nearly contact. *Tawa* shares with *Herrerasaurus* pronounced ventral keels on the cervical vertebrae and elongate prezygapophyses in the distal caudal vertebrae, as in neotheropods. The dorsal vertebrae possess hyposphene-hypantra articulations and there appear to be only two sacral vertebrae.

The complete forelimb (found in articulation) and shoulder girdle share numerous apomorphic features with *Herrerasaurus* and neotheropods such as *C. bauri* and *D. wetherilli*. The elongate manus is particularly theropod-like, with metacarpals abutting each other along their shafts (without overlapping margins) and the presence of weak extensor pits (traits also present in the basal ornithischian *Heterodontosaurus*). The shaft width of metacarpal IV is reduced in *Tawa*, and

the accompanying phalanges are greatly reduced. Moreover, digit V is completely absent, as in *Herrerasaurus* and other basal theropods. The manus of *Tawa* retains a plesiomorphically small medial-most distal carpal. The hand of *Tawa* also retains nine carpals, similar to the basal ornithischian *Heterodontosaurus*, whereas *Herrerasaurus* has seven and *C. bauri* has five.

The *Tawa* pelvis is generally plesiomorphic with respect to neotheropods. The preacetabular process of the ilium (GR 241) does not extend anterior to the pubic peduncle. Additionally, the anterior end is rounded, unlike the squared-off morphology of neotheropods. The supraacetabular crest projects laterally without any ventral deflection but is distally restricted in that it does not approach the articular facet of the pubic peduncle. The supraacetabular crest is continuous with the ventrolateral edge of the postacetabular process, as in coelophysoids. In contrast, the pubis displays a well-developed pubic boot similar to that present in neotheropods, *Herrerasaurus*, and *Staurikosaurus*.

The proximal articular sulcus of the femur, common to Dinosauriformes, is asymmetrically developed in *Tawa* and neotheropods. The fourth trochanter is symmetrical and blade-like in lateral outline, in contrast to the plesiomorphic saurischian condition of a thick asymmetrical ridge. The proximal condyles of the tibia align along the posterior

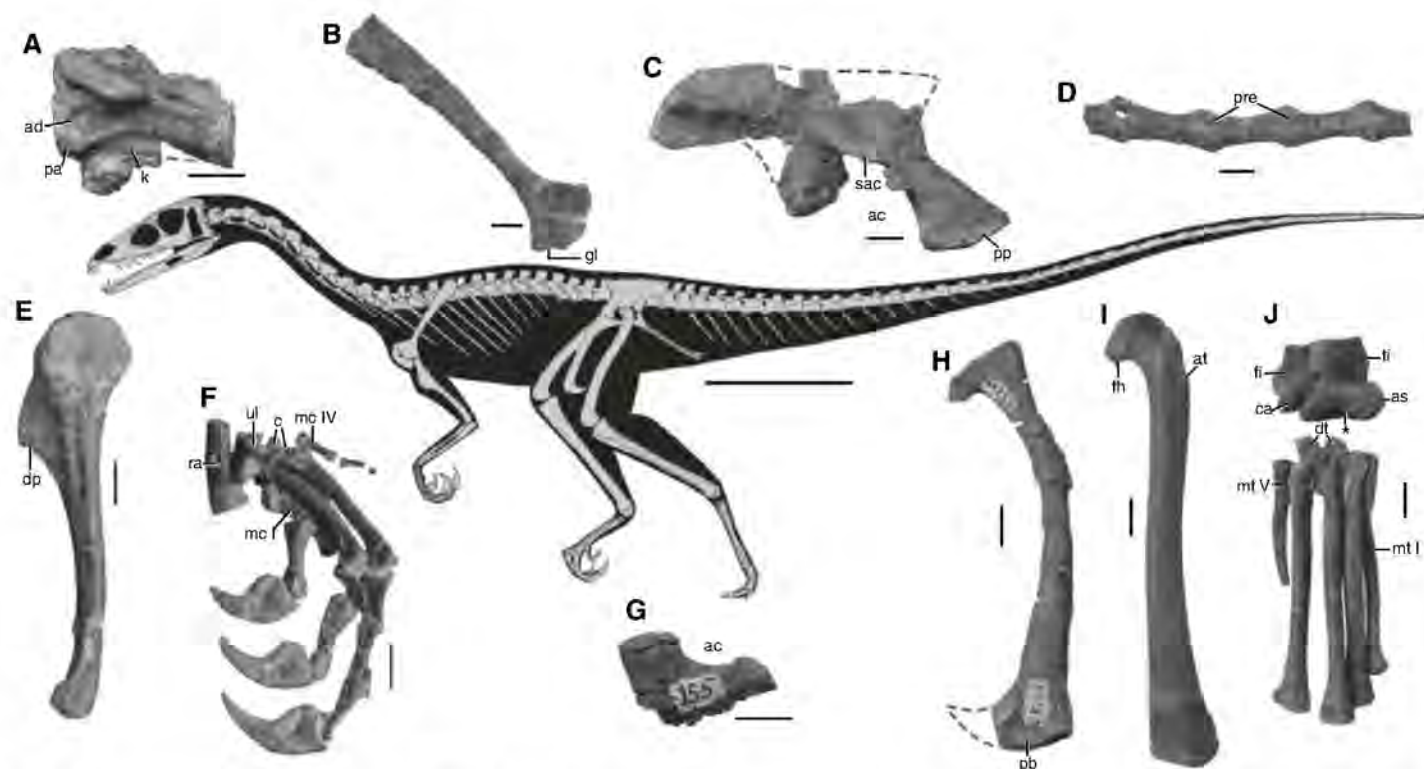


Fig. 2. Skeletal anatomy of *T. hallae* nov. taxa. (A) Anterior cervical vertebra (GR 243) in lateral view. (B) Right scapula (GR 242) in lateral view. (C) Right ilium (GR 155) in lateral view. (D) Middle caudal vertebrae (GR 155) in lateral view. (E) Left humerus (GR 242) in lateral view. (F) Complete right manus (GR 242) in posterior view. (G) Right proximal portion of the ischium (GR 155) in lateral view. (H) Right pubis (GR 155) in lateral view. The proximal portion of the apron is incomplete. (I) Left femur (GR 244) in anterior view. (J) Articulated right pes (GR 242) in anterior view.

Abbreviations in the figure are as follows: acetabulum (ac), anterior depression (ad), astragalus (as), anterior trochanter (at), carpals (c), calcaneum (ca), deltopectoral crest (dp), distal tarsals (dt), femoral head (fh), fibula (fi), glenoid (gl), keel (k), metacarpal (mc), metatarsal (mt), parapophysis (pa), pubic boot (pb), pubic peduncle (pp), prezygapophyses (pre), radius (ra), supraacetabular crest (sac), tibia (ti), and ulna (ul). Matrix was digitally erased from around the manus. Scale bars, 1 cm and reconstruction scale = 0.25 m. Autapomorphies are noted by an asterisk.

edge as in *Herrerasaurus* and neotheropods (such as *C. bauri*). The tibia lacks a fibular crest, and the cnemial crest is not proximally expanded above the proximal articular surface. Two neotheropod character states—an expanded medial edge and a distinct proximodistally elongate posterior ridge—are absent on the distal end of the tibia in *Tawa*. The pes of *Tawa* is plesiomorphic in having metatarsals I to IV elongated. As in other basal saurischians, the fourth tarsal lacks a rounded posterior edge and the astragalus and calcaneum are not co-ossified. The astragalus retains a rimmed basin on the proximal surface posterior to the ascending process. However, the calcaneum of *Tawa* is reduced relative to the astragalus in a manner similar to that of neotheropods. It is mediolaterally compressed and completely lacks a medial process. Metatarsal I retains contact with the ankle in *Tawa*, as in *Herrerasaurus* and *Eoraptor*.

Cladistic analysis identifies *T. hallae* as the closest taxon to Neotheropoda (Fig. 3). The transitional morphology of *Tawa* present in both the skull and the postcranium results in the recovery of *Herrerasaurus* and *Eoraptor* as definitive basal theropods. Although initially described as early theropods (1, 2), the phylogenetic affinities of these taxa have been debated, with some authors arguing for a nondinosaurian position for *Herrerasaurus* (13), a nontheropod, but basal saurischian position for *Herrerasaurus* and *Eoraptor* (14), a basal saurischian position for *Herrerasaurus* and a theropod position for *Eoraptor* (15), or a basal theropod position for both taxa (5). In our analysis, *Herrerasaurus* forms a monophyletic Herrerasauridae with *Staurikosaurus* and *Chindesaurus* at the base of Theropoda, although clade support is weak (10). It takes six steps to recover *Tawa* and *Chindesaurus* as sister taxa. *Eoraptor* and *Tawa* form successively closer sister taxa to Neotheropoda.

Despite the absence of postcranial skeletal pneumaticity in the basal saurischians *Saturnalia*, *Herrerasaurus*, and *Eoraptor*, the presence of anterior cervical pleurocoels in *Tawa* and *Chindesaurus* supports the hypothesis that the origin of cervical air sacs predates the divergence of Neotheropoda and may be ancestral for Saurischia or possibly even Ornithodira [(16) and references therein]. The disarticulated braincase of the holotype of *Tawa* also documents the earliest example of an expansive pneumatic anterior tympanic recess, and the caudal expansion of this recess into the basioccipital. The weak excavation on the ventral surface of the basisphenoid in *Tawa* relative to neotheropods also suggests that development of the cavities associated with the middle ear sac (such as the anterior tympanic recess) preceded the elaboration of the median pharyngeal system into an expansive basisphenoid sinus in neotheropods. Despite the extensive nature of the anterior tympanic recess of *Tawa*, an antero-medial border to the recess is still provided by the basisphenoid and prootic. This reinforces the hypothesis that contralateral connections of the tympanic diverticula are not homologous in crocodiles

and birds, and that the “interaural passage” used in sound localization by modern avians, and possibly some basal coelurosaurs (17), had not evolved by the time of the divergence of *Tawa* from Neotheropoda.

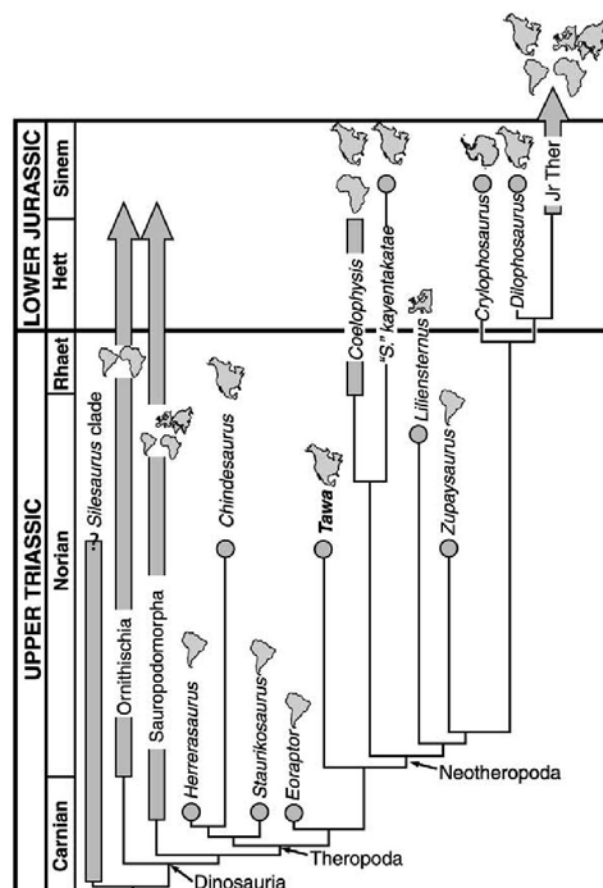
Coelophysoid monophyly is unsupported in our analysis. Without *Tawa* and *Eoraptor*, phylogenetic analyses support a variety of characters as synapomorphies of a clade of “coelophysoid” taxa (*Coelophysis*, “*Syntarsus*” *kayentakatae*, *Liliensternus*, *Zupaysaurus*, *Cryolophosaurus*, and *Dilophosaurus*), because they are absent in both tetanurans and neotheropod outgroups (10). With *Tawa*, these characters are more clearly inferred to have been primitive for Neotheropoda and later lost in the lineage leading to Tetanurae (10). Previous work (18) suggested that resolution of an inclusive Coelophysoidea may be artificial for two reasons: (i) the failure to sample Early Jurassic taxa that possess a mosaic of coelophysoid and more-derived neotheropod features, and (ii) the failure to recognize a broader distribution among basal dinosaurs of many coelophysoid synapomorphies. *Tawa* confirms these hypotheses because it possesses both coelophysoid traits (such as the low angle of the narial process to the premaxillary alveolar margin, the presence of a subnarial gap, and a strong ridge connecting the supraacetabular shelf and brevis shelf) and neotheropod plesiomorphies that collapse Coelophysoidea in our analysis (10). We suggest that the traditional basal theropod clade Coelophysoidea has acted as a phylogenetic vacuum cleaner, with deep thermo-

pod synapomorphies and ceratosaur/tetanuran reversals being “sucked up” and optimized as coelophysoid synapomorphies, because critical taxa were absent across the basal theropod tree. This result reiterates the centrality of new discoveries and increased taxon sampling to providing increased phylogenetic accuracy by breaking long branches [see references in (19)], is critically important for polarizing character evolution in more-derived theropod lineages, and bears directly on the magnitude of turnover in theropod faunas at the Triassic-Jurassic and Early-to-Middle Jurassic boundaries (7, 20).

The presence of multiple carnivorous theropod lineages (*Chindesaurus*, a coelophysoid-grade theropod, and *Tawa*) and an absence or rarity of sauropodomorphs suggest that the HQ saurischian assemblage was qualitatively more like that of the older Ischigualasto Formation (21), where only a single sauropodomorph specimen has been reported, than that of the overlying Los Colorados Formation, which is closer in age to the Hayden assemblage (21). In contrast, the Los Colorados saurischian assemblage contains diverse and abundant sauropodomorphs but only a single reported theropod. These patterns support the hypothesis that the evolution of Triassic dinosaur faunas was diachronous across Pangea (12).

The HQ taxa are spread throughout the stem of theropod phylogeny, and none are each other's closest relative. This demonstrates that they do not represent a monophyletic Norian radiation endemic to the North American protocontinent. Instead, the

Fig. 3. Phylogenetic relationships of *T. hallae* among dinosaurs and the paleobiogeographic distribution of early dinosaur taxa [see SOM (10) for details of the analysis]. Relative temporal relationships for the early Mesozoic are indicated, with minimum ghost lineage extensions implied by phylogeny. The length of the gray bars indicates stratigraphic imprecision, and those with arrows continue through the Sinemurian. Abbreviations are as follows: Hett, Hettangian; Jr Ther, other Jurassic theropods; Rhaet, Rhaetian; and Sinem, Sinemurian.



HQ theropods are separated from each other by branches subtending taxa from other continental faunas, indicating that dispersal between these geographical regions probably occurred during the Camian-Norian. Other contemporaneous theropod assemblages from Europe (22) and South America contain only members of Neotheropoda and do not match the diversity of theropods at the HQ.

Both parsimony (23) and likelihood-based (24) biogeographic methods for ancestral range reconstruction reject scenarios of an endemic North American theropod radiation (10). Analyses differ slightly in support for range reconstructions at individual nodes, but provide high relative support for inferring the South American protocontinent as the ancestral range through the spine of the basal dinosaur tree (10). In most analyses, the distributions of the three HQ theropods are explained by either dispersal to North America from South America or allopatric and/or vicariant speciation from an ancestral widespread range encompassing North and South America (10). This pattern is apparent in many other clades during the Late Triassic, including aetosaurs (25), crocodylomorphs (26), shuvosaurids (27), and "traversodont" cynodonts (28). The ubiquity of this phylogenetic pattern in clades encompassing markedly different ecomorphotypes argues against the presence of physiographic barriers isolating the Norian faunas of North America. Thus, the conspicuous absence of sauropodomorphs in the Norian of North America (3, 12) cannot be attributed to their inability to disperse to these areas but rather to their inability to become established in areas sampled by Late Triassic terrestrial sedimentary outcrops. Latitudinal differentiation of Norian faunas attributable to climatic

differences and climatic tolerances remains an intriguing explanation for the global ubiquity of basal theropod taxa such as *Tawa* and the North American absence of sauropodomorphs. Indeed, recent paleoclimate models and proxy data for the Late Triassic reveal a marked dichotomy between low and high paleolatitudes (29). Alternative explanations, including smaller-scale ecological differences, community-level interactions, or facies-dependent sampling biases, cannot be ruled out, nor are these explanations mutually exclusive (12). Explaining these patterns remains an outstanding problem in early dinosaur evolution at the nexus of phylogenetic, geologic, and paleoclimatic studies of the Late Triassic.

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An Analytical Solution to the Kinetics of Breakable Filament Assembly

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We present an analytical treatment of a set of coupled kinetic equations that governs the self-assembly of filamentous molecular structures. Application to the case of protein aggregation demonstrates that the kinetics of amyloid growth can often be dominated by secondary rather than by primary nucleation events. Our results further reveal a range of general features of the growth kinetics of fragmenting filamentous structures, including the existence of generic scaling laws that provide mechanistic information in contexts ranging from in vitro amyloid growth to the in vivo development of mammalian prion diseases.

Molecular self-assembly is the basis of phenomena ranging from the construction of materials for nanotechnology (1) to the formation of molecular machineries within living cells (2). The assembly of these frequently complex and highly intricate structures typically depends on a series of individual steps that are inherently simple and are therefore amenable in principle to a quantitative analysis

based on physical principles. An important class of molecular structures that emerges from the self-assembly of simpler components is that of filamentous assemblies of biological macromolecules. Many proteinaceous aggregates of this type, which are increasingly linked with normal and aberrant biological processes (2), form through a nucleation mechanism followed by a self-templated growth where the ends of exist-

ing filaments recruit soluble molecules into aggregates that can themselves multiply through secondary nucleation processes such as fragmentation (Fig. 1A).

One of the key questions in molecular self-assembly phenomena is to determine the relative importance of different microscopic processes and their contribution to the overall reaction (3, 4). Master equation approaches are particularly powerful in this context as they enable the explicit description of microscopic processes and have thus offered a series of insights (5–10) into phenomena including the formation of amyloid fibrils, species that are of increasing interest particularly because of their association with clinical disorders ranging from Alzheimer's disease to type II diabetes (2). The lack of analytical

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solutions to such master equations has, however, represented a challenge in the quest to establish general principles and laws governing filamentous growth. Scaling laws, also known in biology as allometric laws, define the relationships between different properties of a given system, and such laws have enabled a variety of fundamental principles to be revealed in fields ranging from condensed matter physics to ecology and from sociology to economics (11, 12). In this work, we illustrate how the availability of an analytical treatment of a master equation (Eq. 1) enables the rationalization of a wide range of experimental results relating to the self-assembly processes of peptides and proteins based on scaling laws and increases the predictive power of the analysis of such phenomena.

The basic processes that enter the master equation describing filamentous protein aggregation have been established previously. Amyloid formation has almost universally been shown to involve a nucleation-dependent polymerization reaction (13), where the formation of growth nuclei from soluble proteins is slower than the elongation of preformed nuclei. In addition, secondary nucleation mechanisms (3) have been identified (5, 7, 14) that lead to the formation of additional nuclei from preexisting filaments; we include here a common type of such mechanism, namely filament fragmentation (5), and other types can also readily be considered within the same scheme [see Supporting Online Material (SOM)]. These molecular processes lead to a master equation describing the time evolution of the concentration $f(t, j)$ of filaments of length j in the system as an infinite set of coupled nonlinear differential equations of the form (6–10)

$$\begin{aligned} \frac{\partial f(t, j)}{\partial t} = & 2m(t)k_+ f(t, j-1) \\ & - 2m(t)k_+ f(t, j) - k_-(j-1)f(t, j) \\ & + 2k_- \sum_{i=j+1}^{\infty} f(t, i) + k_n m(t)^{n_c} \delta_{j, n_c} \quad (1) \end{aligned}$$

where $m(t)$ is the concentration of monomers. The different terms in Eq. 1 represent the elementary microscopic processes. The first term $2m(t)k_+ f(t, j-1)$ accounts for the increase in the number of filaments of length j due to the addition of monomers to either end of a filament of length $j-1$. The term $-2m(t)k_+ f(t, j)$ describes the decrease in their number due to filaments of length j growing further to length $j+1$, the term $-k_-(j-1)f(t, j)$ reflects the possibility of a filament of length j breaking at any of its $j-1$ internal links, and the term $2k_- \sum_{i=j+1}^{\infty} f(t, i)$ accounts for the fact that there are two links in any filament of length $i > j$ where breakage leads to a filament of length j . The last term represents the spontaneous formation of growth nuclei of size n_c as a polynomial form in $m(t)$, which we shall see has the property that it allows the conclusions from the classical theory of homogeneous nucle-

ated growth to be recovered as the limit of our results when setting the fragmentation rate to zero.

Using analytical techniques based on fixed-point mappings (15), we extend linearized early time solutions to describe the full time course of the reaction and reveal [see details in (16)] that the time evolution of the most important experimentally accessible observables, the principal moments of the distribution $f(t, j)$ (the number $P = \sum_{j=n_c}^{\infty} f(t, j)$ and mass $M = \sum_{j=n_c}^{\infty} j \times f(t, j)$ concentrations of filaments) can be written in closed form to a very good approximation (Fig. 1 B) as the integrated rate laws

$$P(t) = \frac{m_{\text{tot}}}{2n_c - 1} - \frac{m_{\text{tot}} k_- e^{-(2n_c - 1)k_- t} \text{Ei}(-C_+ e^{k_- t})}{\kappa} + e^{-(2n_c - 1)k_- t} B_2 \quad (2a)$$

$$M(t) = m_{\text{tot}} [1 - \exp(-C_+ e^{k_- t} + C_- e^{-\kappa t} + k_n m_{\text{tot}}^{n_c - 1} k_-^{-1})] \quad (2b)$$

where $\kappa = \sqrt{2m_{\text{tot}}k_+k_-}$ represents the rate of multiplication of the filament population, $m_{\text{tot}} = M(t) + m(t)$ is the total protein concentration, $\text{Ei}(x) = -\int_{-x}^{\infty} (e^{-y}/y)dy$ denotes the exponential integral function, $C_{\pm} = k_+ P(0)/\kappa \pm M(0)/(2m_{\text{tot}}) \pm (k_n m_{\text{tot}}^{n_c - 1})/(2k_-)$, and the constant B_2 is fixed by the initial condition $P(0)$. We note that the form of the rate of filament multiplication κ —which, as discussed below, is a key parameter in our description of filamentous growth—is consistent with the idea that both the elongation rate $m_{\text{tot}}k_+$ and the fragmentation rate k_- have to double for the overall rate of production of new filaments to double. We point out that we have assumed that the fragmentation rate is independent of the length of the filaments; this assumption was primarily made because of the current difficulty of obtaining reliable experimental estimates of length-dependent fragmentation rates. The approach that we describe, however, can be

in principle modified to take this effect, and indeed others, into consideration when more accurate measurements become available.

In the following, we demonstrate that the theoretical framework provided by Eqs. 2a and 2b has the capacity both to account for and to predict the outcome of protein aggregation reactions of the type associated with protein misfolding diseases. As a first example, we consider the case of a representative protein, insulin; the data in Fig. 2A show that a comprehensive series of reactions performed in vitro with different initial conditions, and resulting in differently shaped kinetic curves ranging from sigmoidal to convex, can be completely accounted for by the solution Eqs. 2a and 2b, with the same values of the two global parameters corresponding to the microscopic elongation and breakage rate constants. According to our treatment, the sigmoidal growth kinetics can be observed as a result of secondary nucleation, here fibril fragmentation, even in the absence of rate-limiting primary nucleation events. This result contrasts with the classical theories of nucleated growth, where homogeneous nucleation is the only source of additional filaments and where such nucleation processes are consequently crucial for determining the duration of the lag phase (13)—the characteristic time of nucleated polymerization reactions during which the initial growth rate is small, and in some cases not measurable using bulk techniques (17).

The filament number concentration $P(t)$ can be reconstructed experimentally (7) from measurements of the free monomer concentration $m(t) = m_{\text{tot}} - M(t)$ and from the measurement of the rate of change $\dot{M}(t)$ in the mass concentration of polymers: $P(t) = \dot{M}(t)/[2k_+ [m_{\text{tot}} - M(t)]]$. Comparison of the results of this reconstruction with the analytical expression for $P(t)$ from Eqs. 2a and 2b demonstrates that in this case also the use of the same values of k_+ and k_- yields good agreement with the measurements (Fig. 2B, inset).

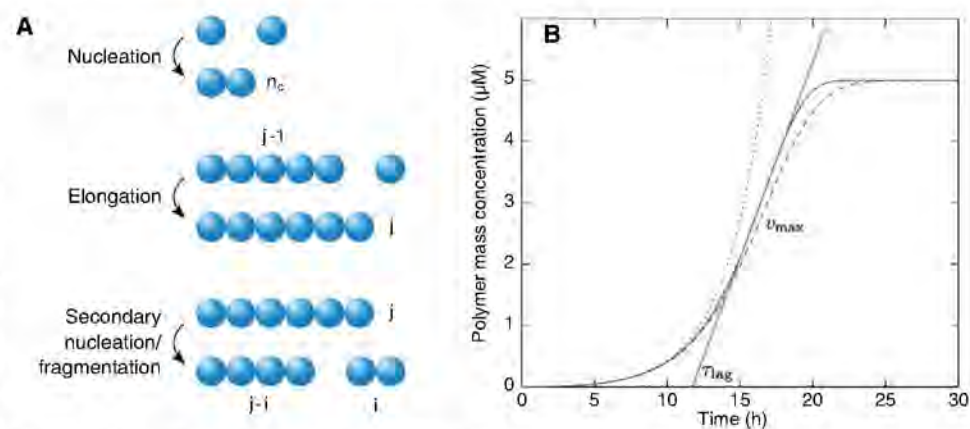


Fig. 1. Kinetics of self-templated aggregation. **(A)** Growth through nucleation (1), elongation (2), and fragmentation (3) leads to sigmoidal kinetic curves **(B)** for the mass concentration of fibrils as a function of time. Dashed blue line, first moment $M(t)$ computed from the numerical solution of the master equation, Eq. 1; solid blue curve, analytical solution given in Eqs. 2a and 2b, obtained through fixed-point iteration of the early time limit (dotted curve). The parameters are $k_+ = 5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, $k_- = 2 \times 10^{-8} \text{ s}^{-1}$, $m_{\text{tot}} = 5 \times 10^{-6} \text{ M}$, $k_n = 2 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$, $n_c = 2$, and $M(0) = P(0) = 0$. The definitions of the lag phase τ_{lag} and the maximal growth rate v_{max} are shown.

The importance of fibril fragmentation for the phenomenon of amyloid growth can be illustrated with the very high sensitivity of the growth kinetics to mechanical shear. We examine in this context the polymerization of β -lactoglobulin (18) under a series of different controlled rates of shearing that promote breakage. We considered a constant primary nucleation rate, k_n , for the entire data set, and a one-parameter fit to each kinetic curve to allow the breakage rate to vary through different values of κ . As shown in Fig. 2C, the variation of this single parameter can account explicitly for the observed differences in both the lag time and the growth rate under different shear conditions. This result shows that even a moderate increase in fracture rate can have very important consequences for the number of protein molecules incorporated into the aggregates; hence, fragmentation emerges as possessing fundamental importance for determining key observables such as the lag phase. This result is also likely to lie at the heart of the well-known high sensitivity of the kinetics of fibril growth to agitation or sonication, processes known to introduce enhanced fragmentation but likely to leave largely unaffected other rate constants for the system.

Based on knowledge gained from the analysis of the rates of the individual processes underlying the growth of protein fibrils, we can now predict

quantitatively how the course of this reaction is affected by changes in system parameters such as protein concentration. To illustrate this idea, we consider data from (19) for the amyloid growth rates of the WW domain in vitro as the concentration of the monomeric protein is varied over an order of magnitude. We extract the microscopic rates through a fit to one of the kinetic curves (Fig. 2D, blue line), and then quantitatively predict as a function of time, with no free parameters, the behavior of the system for a different set of initial conditions (Fig. 2D, gray line) simply through the scaling of the microscopic rates with concentration according to Eqs. 2a and 2b.

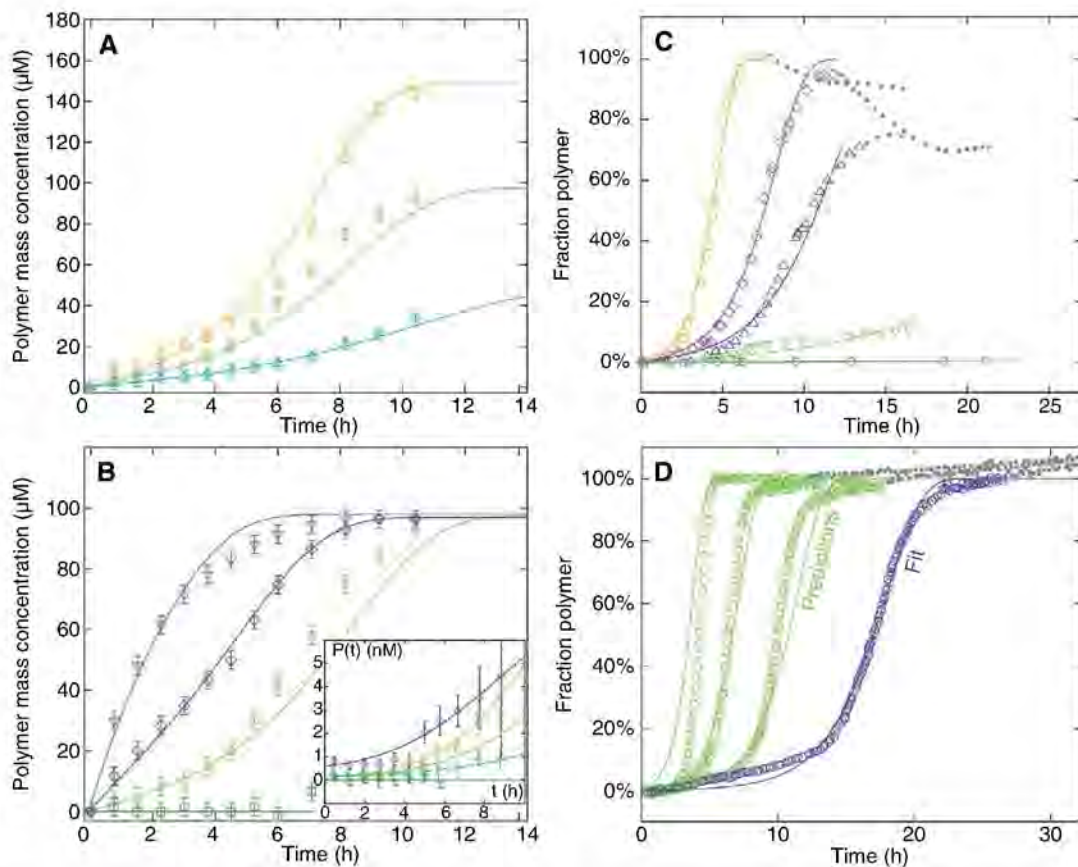
Our analytical theory further reveals general features of the growth of fragmenting filaments. First, considering the normalized maximal growth rate, given as the steepest slope of the sigmoidal kinetic trace (Fig. 1A) divided by the initial soluble protein concentration $v_{\max} = m_{\text{tot}}^{-1} \max_t [\dot{M}(t)] \approx m_{\text{tot}}^{-1} [\dot{M}(t)]_{t=t_{\max}} = \kappa/e$, where $t_{\max} = \kappa^{-1} \log(1/C_+)$ and we have kept the leading C_+ term in Eqs. 2a and 2b over constant and decaying terms, our results show that this maximal rate depends on the nature of the aggregates and the environment in which they are formed only through the single parameter κ , and not on other system parameters such as the number of nuclei present initially or on the primary nucleation rate. This re-

sult is in contrast to classical linear growth theories (20), where the growth rate and lag phase have strong dependencies on the specific details of the homogeneous nucleation process.

A closer examination of the role of the parameter κ reveals that it also essentially defines the lag phase. The lag phase exists only if the growth rate $m_{\text{tot}}\kappa/e$ is maximal at the inflection point $t_{\max} = \log(1/C_+)\kappa^{-1}$, or equivalently for $M(0) < M_C = \kappa L_0/(2k_+e)$, where M_C represents a critical seed concentration which, if exceeded, results in a reaction proceeding without a lag phase. This prediction for the existence of such a threshold can be verified experimentally as shown in Fig. 2B. The conditions used result in a threshold of $M_C = 0.9 \mu\text{M}$, and it can be seen that the reaction starting with $M(0) = 0.21 \mu\text{M}$ proceeds with a marked time lag, whereas for $M(0) = 2.4 \mu\text{M}$ the rate profile is convex. If we define the lag time as shown in Fig. 1A by extrapolation from the maximal growth rate, we obtain the expression $\tau_{\text{lag}} = [\log(1/C_+) - e + 1]\kappa^{-1}$.

It is well known that primary nucleation is an essential step in the phenomenon of amyloid growth in the absence of seeds, and a contribution to the duration of the lag phase can indeed result from such a nucleation process. Our results show, however, that the nucleation rate enters only as a logarithmic correction through C_+ .

Fig. 2. Experimental measurements of the polymer mass concentration $M(t)$ of fibrillar insulin in (A) and (B) are explained using Eqs. 2a and 2b with the two parameters $k_+ = 2.9 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ and $k_- = 2.1 \times 10^{-9} \text{ s}^{-1}$. In (A), the total monomer concentration was (from top to bottom) $m_{\text{tot}} = 149 \mu\text{M}$, $98 \mu\text{M}$, and $49 \mu\text{M}$, and the seed mass concentration used was $M(0) = 0.21 \mu\text{M}$. In (D), for a constant $m_{\text{tot}} = 98 \mu\text{M}$, the seed concentration was (from left to right) $M(0) = 2.4 \mu\text{M}$, $0.8 \mu\text{M}$, $0.21 \mu\text{M}$, and $0 \mu\text{M}$, and the average polymerization number of the seed fibrils $L_0 = M(0)/P(0) = 1380$ was estimated from AFM measurements (see SOM). The inset in (B) shows the polymer number concentration $P(t)$ determined simultaneously for the data in (A) and (B) for the same values of k_+ and k_- . The effect of fragmentation is shown in (C); the polymerization of β -lactoglobulin was measured by Hill *et al.* (18) for increasing shear rates (right to left: 0, 25, 50, 100, and 200 s^{-1}) and was scaled here between 0% and 100%. The values $n_c = 2$, $k_+/k_- = 22.8$ were held constant in all the data sets, and $\kappa = 1.2 \times 10^{-4} \text{ s}^{-1}$, $4.3 \times 10^{-5} \text{ s}^{-1}$, $2.6 \times 10^{-5} \text{ s}^{-1}$, $3.7 \times 10^{-6} \text{ s}^{-1}$, and 0 s^{-1} . (D) Experimental measurements from Ferguson *et al.* (19) for the polymerization of the WW domain. The two parameters in Eqs. 2a and 2b were derived from the data at $m_{\text{tot}} = 50 \mu\text{M}$ (blue curve); using the resulting values of $k_+k_- = 1.7 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-2}$, $k_+/k_- = 13.2 \text{ M}^{-1}$ to predict the polymerization time course for $m_{\text{tot}} = 100 \mu\text{M}$, $200 \mu\text{M}$, and $500 \mu\text{M}$ is shown in gray, whereby the parameter $n_c = 2$ is fixed.



The comparison between this prediction (green curves) and the experimental measurements (green circles) demonstrates that both the maximal rate and the time at which this rate occurs are predicted accurately. Data not considered in the fits are shown with filled gray squares.

in the expression for τ_{lag} ; therefore, when secondary nucleation pathways are active, we conclude that the experimental lag time is primarily determined by the exponential growth regime that takes place in the initial phase of the reaction. Under these circumstances, the formation of oligomeric species, a process that has been observed concomitantly with fibril formation and linked

with cellular toxicity (2), is likely to contribute to such logarithmic terms and may therefore have a limited influence on the overall growth kinetics of fibrillar species. These results reveal that, although the kinetic curves for processes dominated by primary nucleation (20) and by secondary nucleation give rise to apparently similar kinetic traces, their origin is fundamentally different.

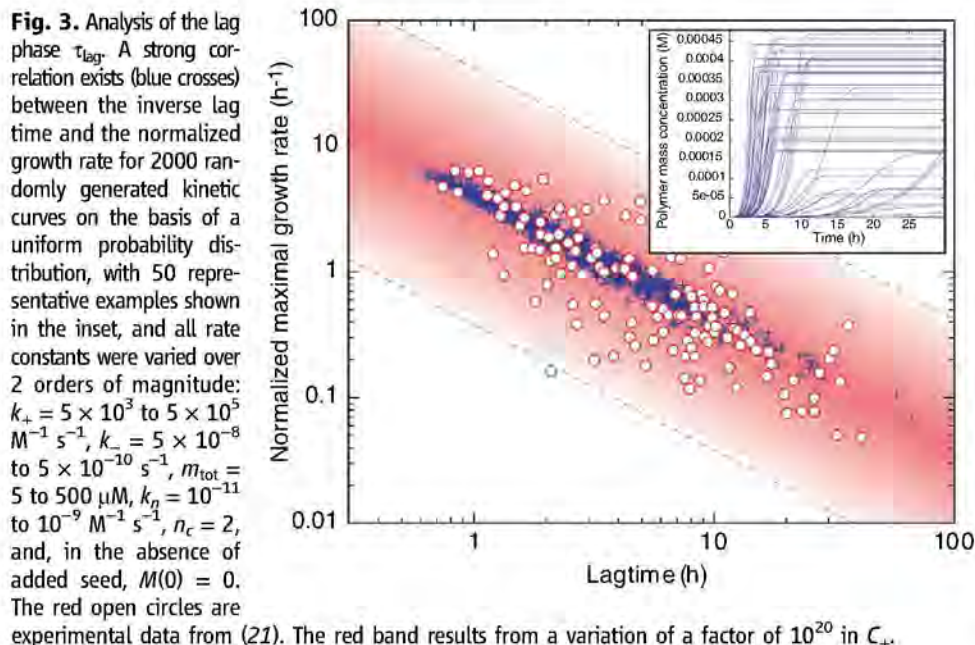


Fig. 3. Analysis of the lag phase τ_{lag} . A strong correlation exists (blue crosses) between the inverse lag time and the normalized growth rate for 2000 randomly generated kinetic curves on the basis of a uniform probability distribution, with 50 representative examples shown in the inset, and all rate constants were varied over 2 orders of magnitude: $k_+ = 5 \times 10^3$ to 5×10^5 $\text{M}^{-1} \text{s}^{-1}$, $k_- = 5 \times 10^{-8}$ to 5×10^{-10} s^{-1} , $m_{\text{tot}} = 5$ to 500 μM , $k_n = 10^{-11}$ to 10^{-9} $\text{M}^{-1} \text{s}^{-1}$, $n_c = 2$, and, in the absence of added seed, $M(0) = 0$. The red open circles are experimental data from (21). The red band results from a variation of a factor of 10^{20} in C_+ .

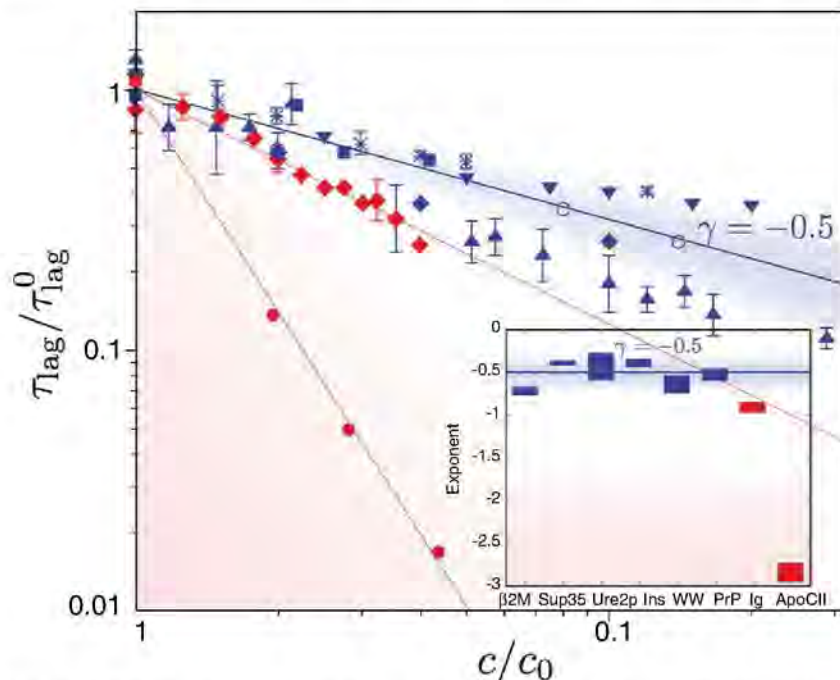


Fig. 4. Scaling of the lag phase with protein concentration. Data are shown for $\beta 2$ -microglobulin ($\beta 2\text{M}$) (8) (blue upward triangles); the yeast prion fragment Sup35 (7) (blue crosses); Ure2p yeast prion (22) (blue squares); insulin (27) (blue downward triangles); WW domain (19) (blue diamonds); TI I27 immunoglobulin domain (Ig) (29) (red diamonds); apolipoprotein C-II (ApoCII) (28) (red circles); and times for onset of terminal disease in 50% of mice inoculated with a fixed dose of prions at $t = 0$ (PrP) (26) (blue circles). The slope corresponding to the exponent $\gamma = -0.5$ is shown with a blue solid line, and fits to the data sets with $\gamma < -0.5$ are shown in red. The inset shows the scaling exponents determined from the data in the main figure for the different systems. The box height in the inset shows standard errors from the linear fit.

Our theory also provides a general demonstration of the validity of a conjecture about amyloid fibril growth (21, 22) that the lag time is commonly highly correlated with the inverse maximal growth rate. Indeed, for fragmentation-assisted growth, we have shown that both the lag phase and the maximal growth rate depend primarily on κ . Therefore, the correlation emerges naturally without the requirement for the responses of the processes of nucleation and growth to changes in system parameters to be identical, a situation generally likely to be incompatible with structural and mechanistic information but required to enforce this correlation under the assumption that the lag phase is defined by primary nucleation processes and the growth phase solely by fibril elongation. Although the coefficient $[\log(1/C_+) - e + 1]$ linking the lag time to the maximal growth rate is variable for different proteins and different experimental conditions, the fact that these differences only enter as logarithmic corrections implies that the general inverse correlation between lag time and growth rate remains valid. In Fig. 3, this idea is illustrated with a red band that shows that the overall correlation is maintained, even when the factor C_+ has been artificially varied by a factor of 10^{20} , a value chosen to exceed greatly the range of variability accessible experimentally.

More generally, the parameter κ , which corresponds to the rate of multiplication of the population of filaments, emerges as the most important quantity describing the overall properties of systems that self-assemble by processes that involve elongation and fragmentation. Our results show that in the regime where secondary nucleation through fragmentation of aggregates is a more effective source of additional structures than primary nucleation [$k_-/(k_n m_{\text{tot}}^{n_c-1}) \gg 1$], observables such as the lag time and maximal growth rate depend primarily on just the single parameter κ .

A particularly striking result that follows from the dominance of a single kinetic parameter in this regime of elongation-fragmentation growth is that generic scaling laws emerge for the behavior of the system. For instance, the lag time is predicted to scale weakly with an exponent $\gamma = -0.5$ with respect to the monomer concentration (because $\tau_{\text{lag}} \sim \kappa^{-1} \approx m_{\text{tot}}^\gamma$ with $\gamma = -0.5$). We have analyzed experimental results for eight unrelated systems, including short peptides, proteins such as insulin and $\beta 2$ -microglobulin, and different types of yeast prions, and find that five of them exhibit this type of weak scaling with high accuracy (Fig. 4). Interestingly, in absolute value, this exponent, 0.5, is crucially smaller than the value of 1.0 or greater required by traditional models (20) in which primary nucleation is a dominant effect determining the duration of the lag phase. In these latter models, the lag time scales with an exponent $\gamma = -(n_c + 1)/2$, which is always $|\gamma| \geq 1$ for a nucleus $n_c \geq 1$ (20), a result that is clearly inconsistent with the experimental observations for the majority of systems we have

evaluated (Fig. 4). Furthermore, in the case of the aggregation of glutamine-rich peptides, nucleus sizes of less than 0 appear to emerge (23) when using classical theories of homogeneous nucleated growth, whereas this type of low concentration dependence emerges naturally from our less-than-linear scaling laws and suggests that breakage is likely to be a key factor in the aggregation of polyglutamine peptides and hence potentially an important contributor to the development of disorders such as Huntington's disease (2).

It is interesting to note that in the limit of a vanishing breakage rate, $k_- \rightarrow 0$, an expansion of the exponentials in Eqs. 2a and 2b up to quadratic order in κ yields a polynomial growth form $M(t) \approx M(0) + k_+ k_n m_{\text{tot}}^{n_c+1} t^2 + 2k_+ m_{\text{tot}} P(0)t + n_c k_n m_{\text{tot}} t$ at the early stages of the reaction. Our result therefore contains in the appropriate limit the behavior characteristic of classical nucleated growth theories (3, 20). It is likely, therefore, that filamentous growth reactions that deviate significantly from the $\gamma = -0.5$ exponent, such as those shown in red in Fig. 4, are limited by complex primary or secondary nucleation processes rather than by simple breakage. For instance, application of the schema described in this paper to growth phenomena where secondary nucleation processes produce additional seeds at a rate that depends on the presence of both filaments and monomer (14) $dP(t)/dt = k_2 M(t)m(t)^{n_2}$ yields a filament mul-

tiplication rate $\kappa = \sqrt{2k_+ k_2 m_{\text{tot}}^{n_2+1}}$ and therefore a

scaling exponent $|\gamma| = (n_2 + 1)/2 \geq 1$ (see SOM), where k_2 represents a rate constant for monomer-dependent secondary nucleation and n_2 is the reaction order of the secondary nucleation.

Finally, we investigate the applicability of the analytical results derived in the present work to in vivo systems. We examine the phenomenon of mammalian prion transmission that is characteristic of disorders such as bovine spongiform encephalopathy and its human analog Creutzfeldt-Jacob disease. The protein-only hypothesis for the propagation of prions describes the conversion of cellular prion proteins (PrP^C) into a pathologically aggregated form (PrP^{Sc}) in a manner that can be transmissible (24). In such cases, PrP^{Sc} aggregates are thought to elongate through the addition of further PrP molecules, and it has been suggested that they multiply rapidly enough through fragmentation of existing structures (5, 25) to allow for transmissibility; these mechanisms are equivalent to the microscopic processes described to the lowest order in Eq. 1. In agreement with this idea, we observe in Fig. 4 that the time for disease onset scales with an exponent $\gamma = -0.52 \pm 0.07$ as a function of the expression level (26) of the prion protein that determines its concentration in vivo; this exponent is fully consistent with the value of -0.5 observed for a range of in vitro systems (Fig. 4) and expected from the analysis developed in this paper for fragmentation-assisted growth, at least when other in vivo limiting factors, such as

cellular clearance mechanisms, have been overwhelmed.

In conclusion, we have provided a unified theoretical framework to address complex biomolecular self-assembly processes that involve primary and secondary nucleation events coupled to linear growth, and demonstrated its value for predicting the kinetics and mechanisms of the proliferation of amyloid fibrils, an example of filamentous growth that is increasingly important in the context of understanding and managing some of the most common and debilitating diseases of the modern era.

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- The kinetic equation for the moment $P(t)$ can be found by taking the sum over j from n_c to ∞ on both sides of Eq. 1. The analogous equation for the first moment $M(t)$ is obtained by multiplying both sides by j and then taking the sum. The telescopic sums vanish; rearranging the order in double sums, we obtain, after some algebra, the coupled system

$$\frac{dP(t)}{dt} = k_- [M(t) - (2n_c - 1)P(t)] + k_n m(t)^{n_c} \quad (3a)$$

$$\frac{dM(t)}{dt} = 2[m(t)k_+ - n_c(n_c - 1)k_-/2]P(t) + n_c k_n m(t)^{n_c} \quad (3b)$$

where $m(t) = m_{\text{tot}} - M(t)$, and we set $f(t, j) = 0$ for all $j < n_c$, where $n_c \geq 2$ is the size of the smallest stable aggregate. Eqs. 3a and 3b make intuitive sense, e.g., the number of polymers at a given time, $P(t)$, can increase if, out of $(M - P)$ total links, the chains break at any location more than $(n_c - 1)$ links distant from each end, thereby forming two stable polymers and thus accounting for the $[M - (2n_c - 1)P]$ factor. The general idea underlying the solution of Eqs. 3a and 3b comes from a fixed-point analysis (15). To reformulate the problem in terms of a fixed-point equation, we first formally solve the differential system

$$P(t) = \int_0^t e^{-(2n_c - 1)k_-(t-\tau)} k_- M(\tau) d\tau + \tilde{B}_2 e^{-(2n_c - 1)k_- t} \quad (4a)$$

$$M(t) = m_{\text{tot}} - n_c(n_c - 1)k_-/2k_+ + B_1 e^{-2k_+ t} \int_0^t P(\tau) d\tau \quad (4b)$$

where B_1 and $\tilde{B}_2$ are integration constants, and we have neglected monomer consumption by nucleation terms

$O(k_n)$. We introduce an operator A , acting on the space of the two principal moments and defined by the right-hand sides of Eqs. 4a and 4b; by construction, the fixed points $x^*(t) = [M^*(t), P^*(t)]$ of A with $Ax^* = x^*$ are solutions to Eqs. 3a and 3b to within $O(k_n)$. The fixed-point equation is solved iteratively: $x^* = \lim_{n \rightarrow \infty} A^{(n)}(x_0)$; the resulting solution after one [for $M(t)$] and two [for $P(t)$] iterations is already in excellent agreement with the numerical result (Fig. 1) and with experiments (Figs. 2 to 4). Successful iteration requires a good starting value $x_0(t) = [M_0(t), P_0(t)]$, and we chose for this value the early aggregation limit, when $m_{\text{tot}} - M(t) \approx m_{\text{tot}}$ and Eqs. 3a and 3b give

$$P_0(t) = C_1 e^{\kappa t} + C_2 e^{-\kappa t} - \frac{n_c k_n m_{\text{tot}}^{n_c-1}}{2k_+} \quad (5a)$$

$$M_0(t) = \frac{2k_+ m_{\text{tot}} C_1}{\kappa} e^{\kappa t} - \frac{2k_+ m_{\text{tot}} C_2}{\kappa} e^{-\kappa t} - \frac{k_n m_{\text{tot}}^{n_c}}{k_-} \quad (5b)$$

where the effective rate $\kappa = \sqrt{2k_+ k_- m_{\text{tot}}}$, and we have used the condition $m_{\text{tot}} k_+ \gg k_-$, which guarantees the existence of an initially growing filament population. The constants C_1 and C_2 are related to the initial conditions $C_{1,2} = \frac{1}{2}P(0) + n_c k_n m_{\text{tot}}^{n_c-1}/(4k_+) \pm M(0)\kappa/(4m_{\text{tot}}k_+) \pm k_n m_{\text{tot}}^{n_c}/(4m_{\text{tot}}k_+)$. Using the starting values (5) for the fixed-point iteration, we arrive at the analytical result presented in Eqs. 2a and 2b. Although the agreement between Eqs. 2a and 2b and the numerical solution (Fig. 1) is good (typical differences comparable in magnitude to common experimental errors), systematic differences are present in the intermediate time regime. Qualitatively, these are primarily because the filament number concentration $P(t)$ entering the expression for $M(t)$ in Eqs. 4a and 4b in the first interaction is from the linearized solution and increases more rapidly than the true solution, and therefore the protein conversion rate $dM(t)/dt$ is overestimated. These errors can be minimized by successive iterations of the fixed-point operator beyond the first-order solution discussed here.

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Altered Heterochromatin Binding by a Hybrid Sterility Protein in *Drosophila* Sibling Species

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Hybrid sterility of the heterogametic sex is one of the first postzygotic reproductive barriers to evolve during speciation, yet the molecular basis of hybrid sterility is poorly understood. We show that the hybrid male sterility gene *OdsH* encodes a protein that localizes to evolutionarily dynamic loci within heterochromatin and leads to their decondensation. In *Drosophila mauritiana* × *Drosophila simulans* male hybrids, OdsH from *D. mauritiana* (OdsH_{mau}) acts as a sterilizing factor by associating with the heterochromatic Y chromosome of *D. simulans*, whereas *D. simulans* OdsH (OdsH_{sim}) does not. Characterization of sterile hybrid testes revealed that OdsH abundance and localization in the premeiotic phases of spermatogenesis differ between species. These results reveal that rapid heterochromatin evolution affects the onset of hybrid sterility.

One facet of speciation studies is focused on mechanisms by which populations become reproductively isolated (1). Intrinsic postzygotic isolation results in the sterility or lethality of the F₁ hybrid offspring following a successful fertilization event and the formation of the zygote (2). The Dobzhansky-Muller model [reviewed in (2)] proposes that such reproductive barriers occur due to incompatibilities between genetic loci arising as a by-product of divergence between two populations. The identification of loci involved in F₁ hybrid sterility in the heterogametic sex (XY males or ZW females) is of particular interest, as this defect is postulated to be the earliest postzygotic isolation event to arise between incipient species (2, 3). Yet, the biological basis of the defects that result in hybrid sterility remains largely unknown.

Crosses between *D. simulans* and *D. mauritiana*, which separated ~250,000 years ago (4), produce sterile F₁ hybrid males and fertile females. A series of introgressions of the *D. mauritiana* genome into a *D. simulans* genomic background revealed that the interaction of the *D. mauritiana* X chromosome-encoded OdsH (OdsH_{mau}) protein with the male *D. simulans* genome resulted in male hybrid sterility (5–8). Considerable amino acid divergence was observed between OdsH_{sim} and OdsH_{mau}, especially within the putative DNA-binding homeodomain (16 nonsynonymous and 3 synonymous changes) (5). Homeodomains are characteristic of a well-conserved family of transcription factors regulating early developmental patterning (9). OdsH evolved ~25 million years ago from a gene duplication of *unc-4* (10),

which encodes a transcription factor that has somatic function in *Drosophila* (11).

OdsH expression in the testes (6) and its evolutionary descent from *unc-4* (10) led to the proposal that OdsH encodes a transcription factor whose introduction into the hybrid background causes misexpression of meiotic genes and, therefore, hybrid sterility (12). However, this model fails to account for how the protein-DNA interaction interface may drive the changes observed in the OdsH homeodomain between *Drosophila* species. Ablation of the OdsH gene in *D. melanogaster* had only modest effects on male fertility (6), contrary to expectations that a deletion of OdsH would affect male fertility due to the misregulation of meiotic genes.

An alternative model suggests that evolutionary labile satellite DNAs—found in pericentric, telomeric, and other heterochromatic regions—may result in the divergence of speciation genes (13, 14). Under this model, satellite DNAs and their expansions are perpetuated by female meiotic drive, but affect fitness through reductions in male fertility, which is evident in plant and animal species (15, 16). The evolution of satellite DNA-binding proteins is predicted to be one way to mitigate cost to male fertility and ensure species survival (13, 14). Therefore, we considered the alternative possibility that hybrid sterility genes like OdsH encode proteins that bind to satellite DNA repeats in pericentric or telomeric regions. Under this model, hybrid sterility could result from an inability to correctly package and condense heterochromatin.

To distinguish between euchromatic versus heterochromatic localization, we expressed OdsH_{sim} fused to a 3xFLAG epitope in a *D. simulans* embryonic cell culture line (Fig. 1, A and B). We observed a punctate localization pattern of OdsH_{sim} in interphase cells—reminiscent of the D1 satellite-binding protein (17). In *D. simulans*, D1 predominantly localizes to repetitive satellite sequences on the Y and 4th chromosomes (fig. S1), providing a cytological marker relative

to OdsH localization. On this basis, OdsH_{sim} localized adjacent to D1 in *D. simulans* cells (Fig. 1B). However, the localization of OdsH_{mau} protein (fused to Venus, yellow fluorescence protein) partially overlapped with D1 (Fig. 1, A and C). Coexpression of the OdsH_{sim} and OdsH_{mau} fusion proteins revealed that the two proteins localize to common sites, but that OdsH_{mau} has additional localization (Fig. 1D).

We mapped the chromosomal localization of tagged OdsH_{sim} and OdsH_{mau} proteins with N-terminal 1xFLAG or Venus, respectively. Expression and immunofluorescent detection of OdsH_{sim} in mitotic larval neuroblast cells confirmed that OdsH_{sim} was associated with repeat-rich regions of the *D. simulans* genome, namely, the X pericentric region and the 4th chromosome (Fig. 1E). OdsH_{mau} localized similarly on the *D. simulans* X and 4th chromosomes but showed gross localization to the *D. simulans* Y chromosome (Fig. 1F). Coexpression of OdsH_{sim} and OdsH_{mau} showed the additional localization of OdsH_{mau} to the Y chromosome in male cells (Fig. 1G), whereas these two proteins localized identically in female cells (Fig. 1H). In *Drosophila*, both the Y and 4th chromosomes are principally heterochromatic, gene-poor, and repeat-rich (18). On the basis of these results, we conclude that both OdsH_{sim} and OdsH_{mau} encode heterochromatin-binding proteins but that they have different localization specificities, resulting in altered localization to the *D. simulans* Y chromosome.

Because OdsH shares ancestry with a transcription factor, *unc-4*, we expressed a green fluorescence protein (GFP)-tagged *unc-4* protein to contrast its localization with that of the OdsH protein. As expected for a transcription factor, *unc-4* showed diffuse staining in interphase neuroblast cells (Fig. 1I). Hence, the OdsH protein may have gained specificity for localization to heterochromatin since it diverged from *unc-4*.

We investigated whether divergence of the underlying satellite DNAs was associated with changes in OdsH binding specificity. We identified targets of OdsH binding in sibling species *D. mauritiana* or *D. sechellia* (Fig. 2A and fig. S2) by crossing transgenic *D. simulans* lines expressing fusion proteins of either OdsH_{sim} or OdsH_{mau} to these species. By examining localization in male and female hybrids, we found altered localization of OdsH_{sim} and OdsH_{mau} on the Y and 4th chromosomes of sister species (Fig. 2, B to F, and fig. S3). The *D. sechellia* and *D. simulans* Y chromosomes were enriched for OdsH_{mau} binding, whereas OdsH_{mau} localization to its own Y chromosome was restricted (Fig. 2, E and F). In contrast, OdsH_{sim} did not associate with any of the three Y chromosomes (Fig. 2, C and D). Furthermore, OdsH_{sim} and OdsH_{mau} bound to the 4th chromosome from *D. simulans*, but not from *D. sechellia* or *D. mauritiana* (Fig. 2, B to F). Thus, the localization of OdsH proteins differed on homologous chromosomes from different *Drosophila* species, suggesting that there has been a reorganization or wholesale

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replacement of OdsH-binding sites within heterochromatin on both the Y and 4th chromosomes within the past 250,000 years (summarized in Fig. 2B). These rapid changes in heterochromatin mirror the rapid evolution of OdsH's homeo-domain during this span (5).

Localization of OdsH appears to markedly affect local chromosome condensation. For instance, in *D. simulans* neuroblast nuclei expressing OdsHmau, the general decondensation and intercalation of the Y and 4th chromosomes obscured our ability to distinguish these chromosomes from each other (Fig. 1, F and G, and fig. S4). This is in contrast to nuclei expressing only OdsHsim, in which the Y chromosome is condensed (Fig. 1E). Decondensation of the X and 4th chromosomes was also associated with the localization of either OdsHsim or OdsHmau (Fig. 2, D to F). Decondensation was seen for both OdsHsim and OdsHmau and in all *Drosophila* genomes assayed (fig. S4). It appears that the retention of an ancestral *unc-4*-like transcriptional function, coupled with its localization to heterochromatic regions that are otherwise condensed, has resulted in OdsH-mediated chromosome decondensation in hybrids.

To confirm the localization of OdsH in *D. simulans* cell culture and mitotic chromosomes, we characterized endogenous OdsH local-

ization in the *D. simulans* testis with an antibody raised to the C terminus of OdsHsim (fig. S5). Whole-mount immunohistochemistry (IHC) of OdsH in wild-type *D. simulans* testes showed that OdsH is restricted to developing postmitotic primary spermatocytes in the G₂ phase (Fig. 3A and figs. S7 and S8). Within these spermatocytes, immunofluorescence revealed two punctate dots, consistent with OdsHsim localization to only two major loci within the *D. simulans* genome (Fig. 3G). At this stage of spermatogenesis, homologous chromosomes arrange into individual chromatin domains associated with the nuclear membrane (19). Typically, two large hazy domains represent the associated 2nd and 3rd chromosomes, respectively, while the other two domains represent the associated X-Y and 4th chromosomes (19). The presence of OdsH in the latter chromatin domains suggests that endogenous OdsH binds to loci on the X and 4th chromosomes, consistent with our observations that OdsHsim binds to the X and 4th chromosomes of *D. simulans*.

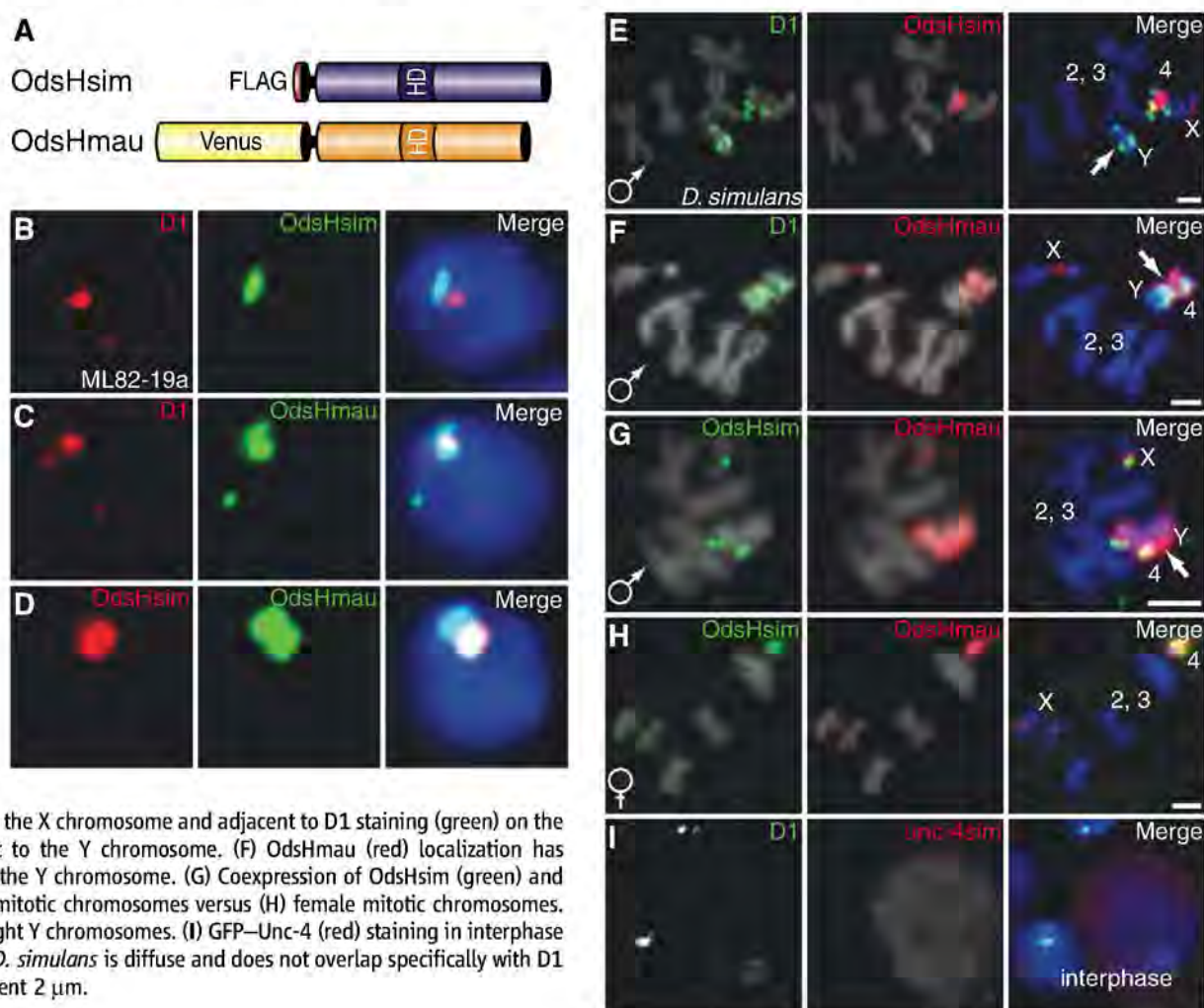
The antibody that we raised to OdsHsim also recognizes OdsHmau with high specificity (fig. S5). Therefore, we investigated OdsH localization using whole-mount IHC on the testes of a *D. simulans* male sterile line, containing an introgression of the *OdsH*-containing region from

the *D. mauritiana* X chromosome (Fig. 3C and fig. S6) (5). Immunofluorescence of OdsH confirmed the localization of OdsHmau to three loci corresponding to the X, Y, and 4th chromosomes within these primary spermatocytes (Fig. 3H). We also observed an expansion of OdsH protein localization in late-G₂ primary spermatocytes of the sterile introgression line as compared to wild-type lines (Fig. 3E), suggesting an increase in protein amounts or stability. Western blot analysis confirmed that OdsH protein abundance was increased in the sterile introgression testes (Fig. 3F). Despite the presence of OdsH protein at this late stage of primary spermatocyte development, we did not observe OdsH protein in spermatocytes that have progressed from G₂ to meiotic prophase or a delay in the onset of meiosis (fig. S8).

A fertile introgression line differing from the sterile line in that exons 3 and 4 of *OdsH* are derived from *D. simulans* (fig. S6) (4) showed no detectable OdsH protein, both by IHC and by Western analyses (Fig. 3, B and F). In addition, we observed no detectable OdsHmau protein in the testes of *D. mauritiana* males (Fig. 3D). Our characterization of endogenous OdsH abundance and localization reveals differences between both sibling *Drosophila* species and the sterile and fertile *D. simulans* introgression lines. Although it is still unclear how the additional binding capacity of

Fig. 1. OdsH proteins differ in their localization to *D. simulans* heterochromatin. We use D1 staining as a marker for 4th and Y chromosome heterochromatin (fig. S1).

(A) FLAG-OdsHsim or Venus-OdsHmau epitope-tagged proteins were expressed in transiently transfected *D. simulans* cultured cells (B to D) or in transgenic *D. simulans* larval neuroblasts (E to H) under the control of a heat-shock promoter. (B and C) D1 staining (red) is a cytological landmark for localization of OdsHsim (B) and OdsHmau (C) proteins (both shown in green) to *D. simulans* heterochromatin. DNA staining by DAPI (4',6-diamidino-2-phenylindole) is shown in blue in merge. (D) Coexpression of OdsHsim (red) and OdsHmau (green). (E) OdsHsim (red) localizes to the X chromosome and adjacent to D1 staining (green) on the 4th chromosome but not to the Y chromosome. (F) OdsHmau (red) localization has additional localization to the Y chromosome. (G) Coexpression of OdsHsim (green) and OdsHmau (red) on male mitotic chromosomes versus (H) female mitotic chromosomes. Arrows in (E) to (G) highlight Y chromosomes. (I) GFP-Unc-4 (red) staining in interphase larval neuroblast cells of *D. simulans* is diffuse and does not overlap specifically with D1 (green). Scale bars represent 2 μ m.



OdsH_{mau} adversely affects premeiotic stages of sperm development to cause male sterility, there is precedent for premeiotic defects manifesting in postmeiotic dysfunction in *Drosophila* and mice (20–22).

Because the studied introgression lines differ only at their OdsH locus, OdsH_{mau} is unambiguously linked to the hybrid male sterility phenotype (5, 6). Our findings reveal the genetic architecture underlying OdsH-mediated hybrid sterility. Both

D. mauritiana and fertile introgressed *D. simulans* males lack detectable OdsH protein, yet are completely fertile, consistent with the fact that OdsH function is not required for male fertility in *D. melanogaster* (6). Thus, OdsH_{mau}-mediated

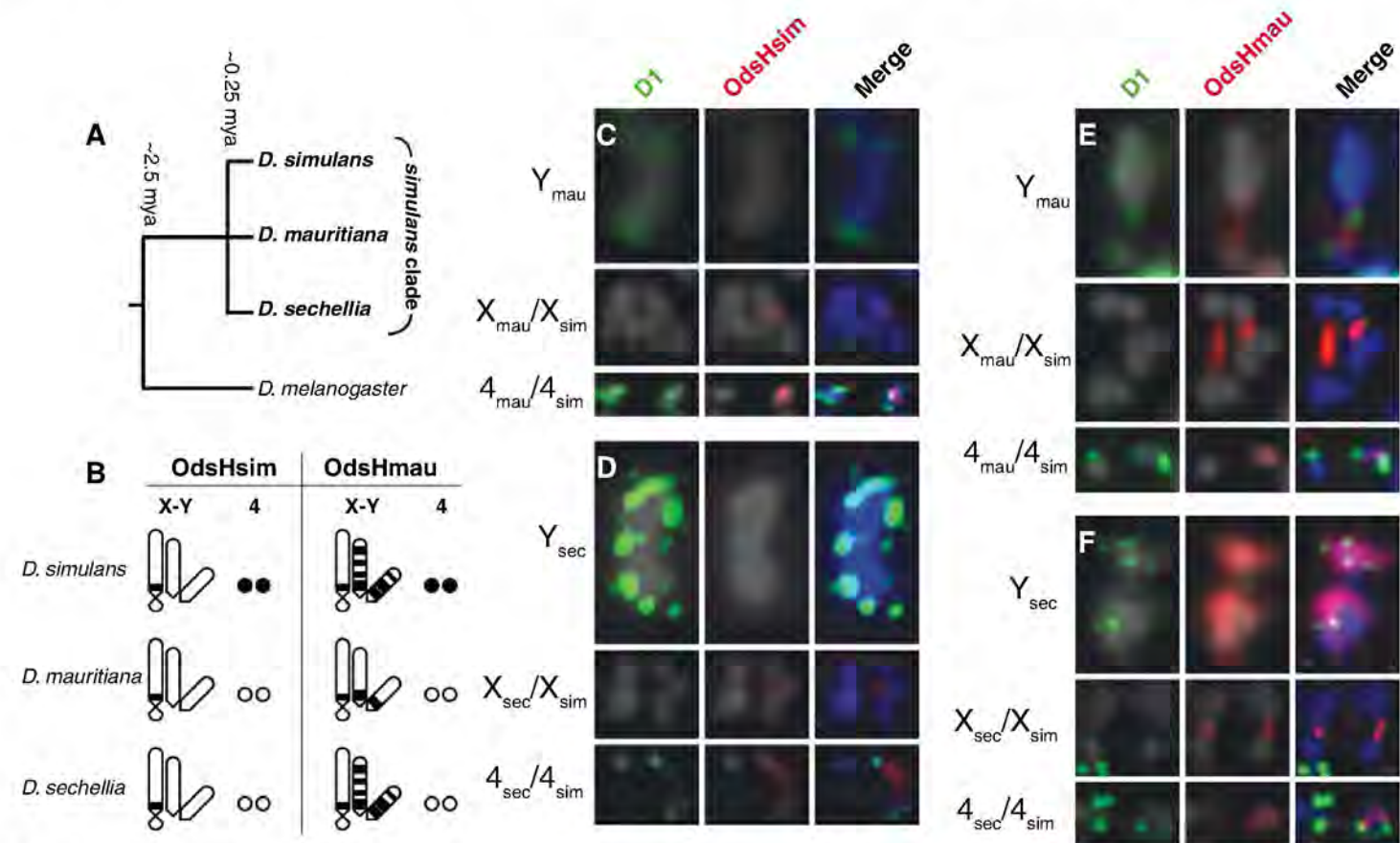
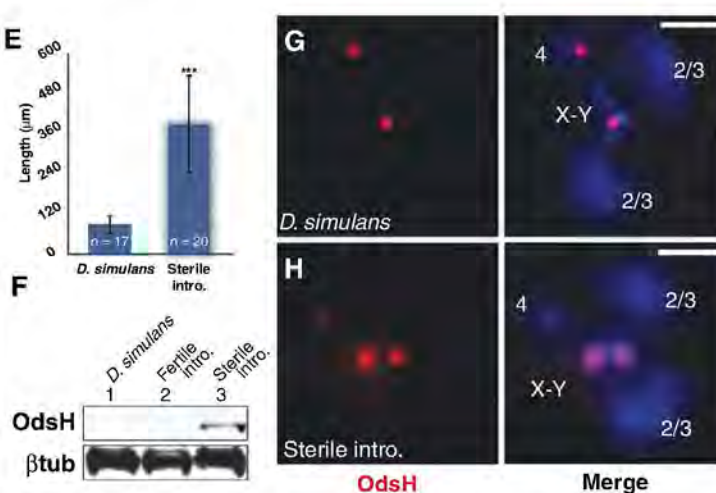
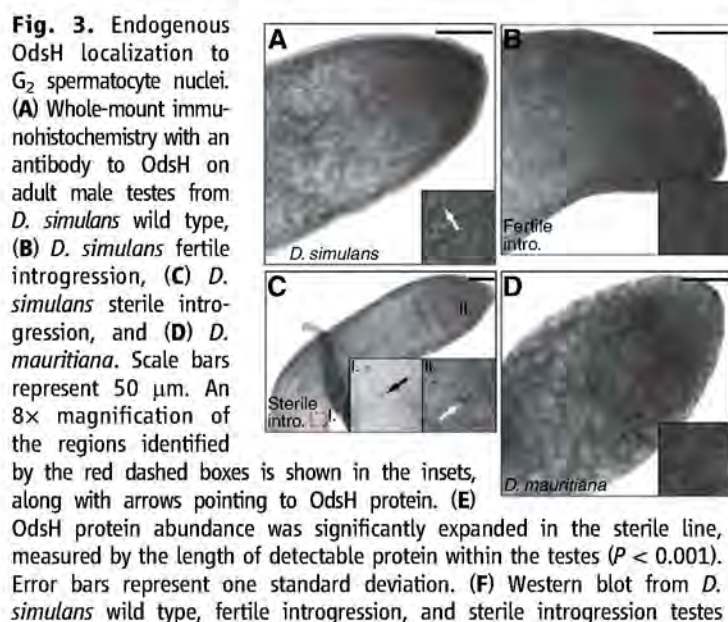


Fig. 2. OdsH-binding sites are evolutionary labile in sibling species. **(A)** A schematic phylogeny of the recently diverged *D. simulans*, *D. sechellia*, and *D. mauritiana* species. mya, millions of years ago. **(B)** Summary of localization studies **(C to F)** highlighting the differences in genomic localization of OdsH_{sim} and OdsH_{mau} to the Y and 4th chromosomes. **(C)** Localization of FLAG-OdsH_{sim} fusion protein on neuroblast mitotic chromosomes from

D. mauritiana/*D. simulans* and **(D)** *D. sechellia*/*D. simulans* hybrid larvae (fig. S2). **(E)** Localization of Venus-OdsH_{mau} fusion protein on neuroblast mitotic chromosomes from *D. mauritiana*/*D. simulans* and **(F)** *D. sechellia*/*D. simulans* hybrid larvae. In all cases, OdsH staining is red and DAPI staining green. Only relevant X, Y, and 4th chromosomes are shown.



immunoblotted for OdsH (top) and β -tubulin control (bottom). **(G)** OdsH (red) detected by immunofluorescence in G_2 primary spermatocyte nuclei from the adult testes of *D. simulans* wild type and **(H)** *D. simulans* sterile introgression. DNA staining is in blue. Scale bars represent 5 μ m.

hybrid sterility involves both the unusual abundance and retention of OdsHmau protein in the *D. simulans* testis, as well as an unusual localization and possibly decondensation of the *D. simulans* Y chromosome. We conclude on the basis of these data that hybrid male sterility is caused by a gain-of-function interaction between OdsHmau and some component of the *D. simulans* Y chromosome heterochromatin, with this protein-DNA interaction representing the Dobzhansky-Muller incompatibility.

OdsH shares similarities with the hybrid sterility genes *Prdm9* (or *Meisetz*) in mouse (23) and *Overdrive* (*Ovd*) in *Drosophila* (24), all of which encode proteins with putative DNA-binding domains. Satellite DNAs have also been implicated in hybrid inviability, including a pericentric satellite locus (*Zhr*) (25, 26) and a gene encoding a heterochromatin-binding protein (*Lhr*) (27). Thus, rapidly evolving repetitive DNA elements driven by genetic conflict may represent a major evolutionary force driving sequence divergence of speciation genes that would ultimately result in hybrid incompatibilities (13, 14, 28).

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Mapping Human Genetic Diversity in Asia

The HUGO Pan-Asian SNP Consortium*†

Asia harbors substantial cultural and linguistic diversity, but the geographic structure of genetic variation across the continent remains enigmatic. Here we report a large-scale survey of autosomal variation from a broad geographic sample of Asian human populations. Our results show that genetic ancestry is strongly correlated with linguistic affiliations as well as geography. Most populations show relatedness within ethnic/linguistic groups, despite prevalent gene flow among populations. More than 90% of East Asian (EA) haplotypes could be found in either Southeast Asian (SEA) or Central-South Asian (CSA) populations and show clinal structure with haplotype diversity decreasing from south to north. Furthermore, 50% of EA haplotypes were found in SEA only and 5% were found in CSA only, indicating that SEA was a major geographic source of EA populations.

Several genome-wide studies of human genetic diversity focusing primarily on broad continental relationships, or fine-scale structure in Europe, have been published recently (1–8). We have extended this approach to Southeast Asian (SEA) and East Asian (EA) populations by using the Affymetrix GeneChip Human Mapping 50K Xba Array. Stringently quality-controlled genotypes were obtained at 54,794 autosomal single-nucleotide polymorphisms (SNPs) in 1928 individuals representing 73 Asian and two non-Asian HapMap populations (9). Apart from developing a general description of Asian population structure and its relation to geography, language, and demographic history, we concentrated on un-

covering the geographic source(s) of EA and SEA populations.

We first performed a Bayesian clustering procedure using the STRUCTURE algorithm (10) to examine the ancestry of each individual. Each person is posited to derive from an arbitrary number of ancestral populations, denoted by *K*. We ran STRUCTURE from *K* = 2 to *K* = 14 using both the complete data set and SNP subsets to exclude those in strong linkage disequilibrium (Fig. 1 and figs. S1 to S13). At *K* = 2 and *K* = 3, all SEA and EA samples are united by predominant membership in a common cluster, with the other cluster(s) corresponding largely to Indo-European (IE) and African (AF) ancestries. At *K* = 4, a component most frequently found in Negrito populations that is also shared by all SEA populations emerges, suggesting a common SEA ancestry. Each value of *K* beyond 4 introduces a new component that tends to be associated with a group of popula-

tions united by membership in a linguistic family, by geographic proximity, by a known history of admixture, or, especially at higher *K*s, by membership in a small population isolate. The results obtained using *frappe* (11), a maximum-likelihood-based clustering analysis, showed a general concordance with those of STRUCTURE (figs. S14 to S26). These analyses show that most individuals within a population share very similar ancestry estimates at all *K*s, an observation that is consistent also with a phylogeny relating individuals (fig. S27) based on an allele-sharing distance (12). Therefore, we proceeded to evaluate the relationships among populations. A maximum-likelihood tree of populations, based on 42,793 SNPs whose ancestral states were known (Fig. 1), showed that all the SEA and EA populations make up a monophyletic clade that is supported by 100% of bootstrap replicates. This pattern remained even after data from 51 additional populations and 19,934 commonly typed SNPs from a recent study were integrated into the tree (fig. S28). These observations suggest that SEA and EA populations share a common origin.

STRUCTURE/*frappe* and principal components analyses (PCA) (13) (Figs. 1 and 2 and figs. S1 to S26) identify as many as 10 main population components. Each component corresponds largely to one of the five major linguistic groups (Altaic, Sino-Tibetan/Tai-Kadai, Hmong-Mien, Austro-Asiatic, and Austronesian), three ethnic categories (Philippine Negritos, Malaysian Negritos, and East Indonesians/Melanesians) and two small population isolates (the Bidayuh of Borneo and the hunter-gatherer Mlabri population of central and northern Thailand). The STRUCTURE results

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(Fig. 1 and figs. S1 to S13), population phylogenies (Fig. 1 and figs. S27 and S28), and PCA results (Fig. 2) all show that populations from the same linguistic group tend to cluster together. A

Mantel test confirms the correlation between linguistic and genetic affinities ($R^2 = 0.253$; $P < 0.0001$ with 10,000 permutations), even after controlling for geography (partial correlation = 0.136; $P <$

0.005 with 10,000 permutations). Nevertheless, we identified eight population outliers whose linguistic and genetic affinities are inconsistent [Affymetrix-Melanesian (AX-ME), Malaysia-Jehai (MY-JH)

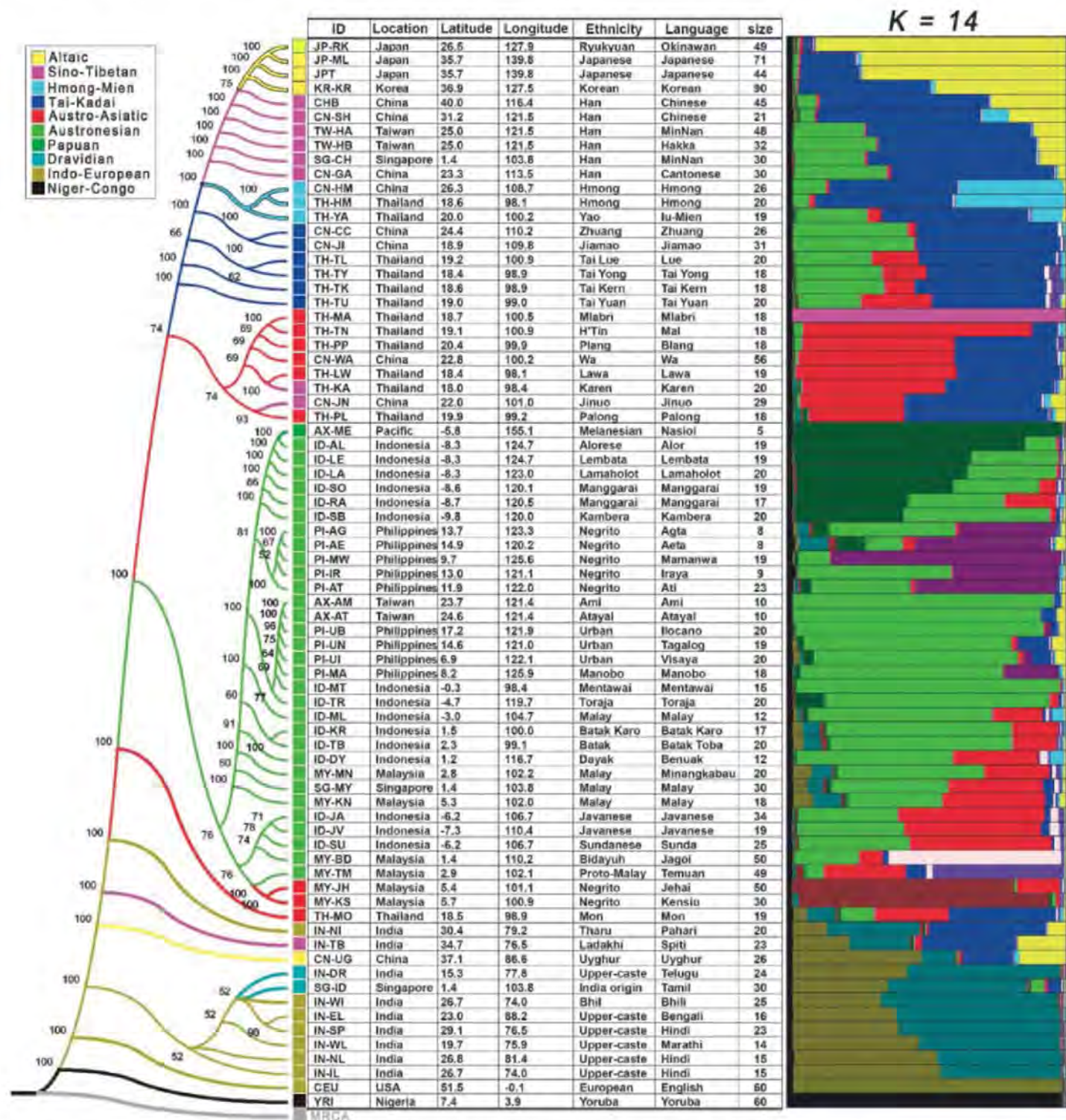


Fig. 1. Maximum-likelihood tree of 75 populations. A hypothetical most-recent common ancestor (MRCA) composed of ancestral alleles as inferred from the genotypes of one gorilla and 21 chimpanzees was used to root the tree. Branches with bootstrap values less than 50% were condensed. Population identification numbers (IDs), sample collection locations with latitudes and longitudes, ethnicities, language spoken, and size of population samples are shown in the table adjacent to each branch in the tree. Linguistic groups are indicated with colors as shown in the legend. All

population IDs except the four HapMap samples are denoted by four characters. The first two letters indicate the country where the samples were collected or (in the case of Affymetrix) genotyped, according to the following convention: AX, Affymetrix; CN, China; ID, Indonesia; IN, India; JP, Japan; KR, Korea; MY, Malaysia; PI, the Philippines; SG, Singapore; TH, Thailand; and TW, Taiwan. The last two letters are unique IDs for the population. To the right of the table, an averaged graph of results from STRUCTURE is shown for $K = 14$.

(Negrito), Malaysia-Kensiu (MY-KS) (Negrito), Thailand-Mon (TH-MO), Thailand-Karen (TH-KA), China-Jinuo (CN-JN), India-Spiti (IN-TB), and China-Uyghur (CN-UG); see table S3]. These linguistic outliers tend to cluster with their geographic neighbors or [especially evident in the principal component (PC) plots of Fig. 2] occupy an intermediate position between their geographic neighbors and the more-distant members of their linguistic group. These patterns are consistent either with substantial recent admixture among the populations (14–16), a history of language replacement (17), or uncertainties in the linguistic classifications themselves (for example, the controversial Altaic family, which groups Korean and Japanese with Uyghur).

Considerable gene flow among Asian populations was observed among subpopulations in these clusters, including those groups believed to

practice endogamy based on linguistic, cultural, and ethnic information. In fact, most populations studied, even at lower K s, show evidence of admixture in the STRUCTURE analyses. For example, the Han Chinese have grown to become the largest ethnic group today in a demographic expansion that has occurred mostly within historical times. STRUCTURE reveals that the six Han Chinese population samples in our study show varying degrees of admixture (Fig. 1 and figs. S1 to S26) between a northern Altaic cluster and a Sino-Tibetan/Tai-Kadai cluster, which most frequently appears in the ethnic groups sampled from southern China and northern Thailand. Finally, most of the Indian populations showed evidence of shared ancestry with European populations, which is consistent with the recent observations (18) and our understanding of the expansion of Indo-

European-speaking populations (Fig. 1 and figs. S1 to S26).

The geographic source(s) contributing to EA populations have long been debated. One hypothesis suggests that all SEA and EA populations derive primarily from a single initial migration, which entered the continent along a southern, largely coastal route (19, 20). Another hypothesis argues for at least two independent migrations into East Asia, first along a southern route, followed later by a series of migrations along a more northern route that served to bridge European and EA populations, but with little contribution to populations in Southeast Asia (20). The topology of a maximum-likelihood tree (Fig. 1 and fig. S28) displays a largely south-to-north ordering of the populations, and a plot of the first two PCs (Fig. 2) similarly orients most populations according to their geographic coordinates. The average

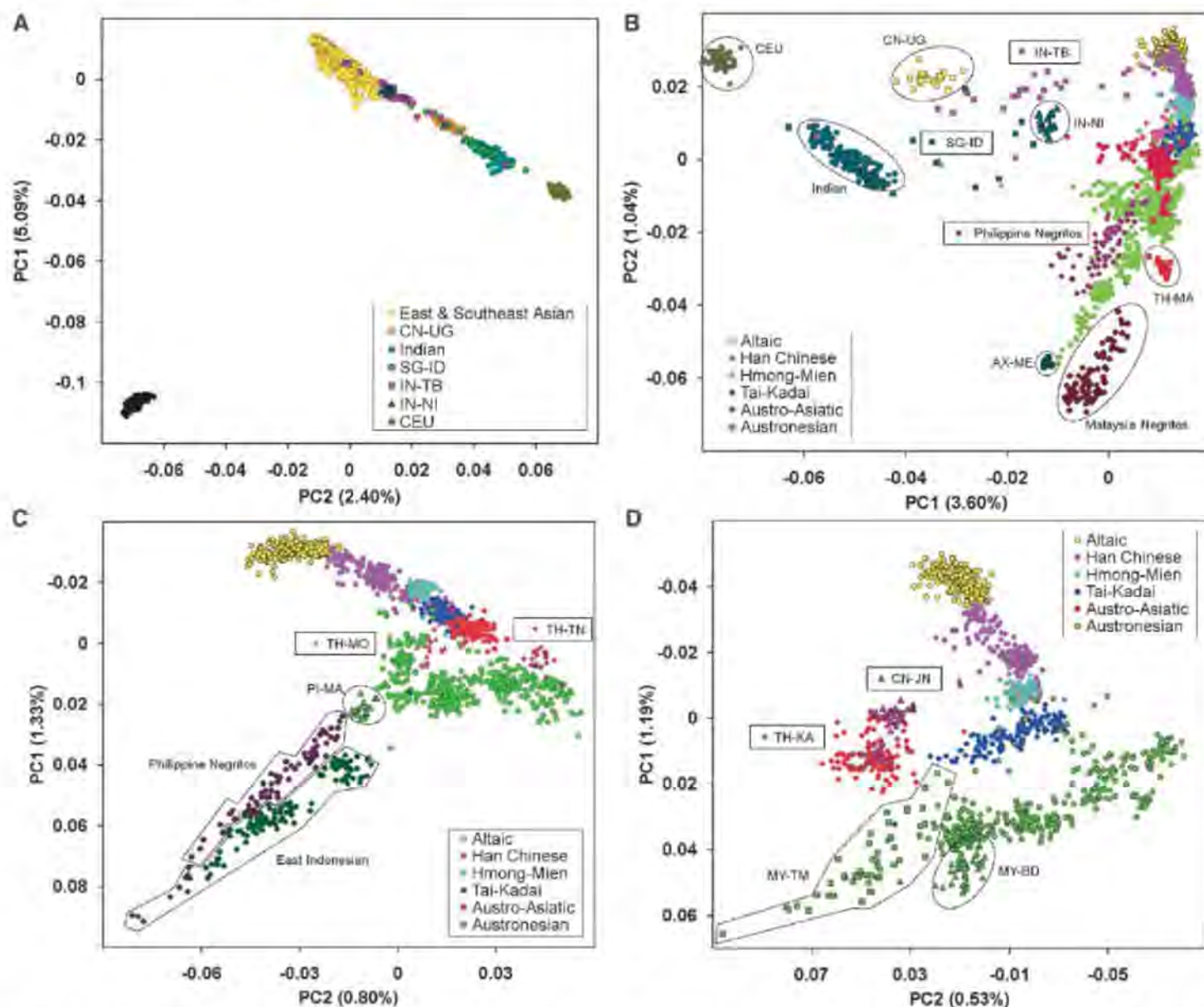


Fig. 2. Analysis of the first two PCs. (A) 1928 individuals representing all 75 populations. (B) 1868 individuals representing 74 populations (excluding YRI). (C) 1471 individuals representing 58 populations (excluding all Indians,

CN-UG, TH-MA, AX-ME, and Negritos from Malaysia). (D) 1235 individuals representing 44 populations (excluding Philippine Negritos, PI-MA, and East Indonesians).

value of the first PC is highly correlated with the latitude at which the populations were sampled ($R^2 = 0.79$, $P < 0.0001$). Such a pattern could result simply from isolation-by-distance (IBD), as suggested by Ding *et al.* (21), although a recent study failed to detect IBD in East Asia with data from the Human Genome Diversity Project (22).

In an effort to distinguish between long-term historical divergence and the effects of IBD, we applied partial and multiple Mantel tests to the data (23) [see supporting online material (SOM) text for details]. The primary approach was to ascertain the differential correlation between genetic distance, geographical distance, and a group indicator matrix as an indication of prehistoric population divergence. The partial correlation coefficient of genetic and geographic distances was 0.228 ($P < 0.0006$), after controlling for the group indicator matrix (inferred from STRUCTURE/

frappe analyses), whereas the partial correlation of the genetic and group indicator matrices was 0.403 ($P < 0.0001$) after controlling for geography. The superior association between genetic distance and the group indicator matrix as measured by the correlation coefficients suggests that prehistorical population divergence is the favored model over IBD in explaining the data (24). This conclusion is supported by simulation studies that also suggest that the observed patterns cannot be explained by simple IBD effects alone (see SOM text for details).

To further refine the analysis, we looked to haplotype organization to limit the effect of fluctuations in single-nucleotide determinations and to increase the resolution around genetic diversity. The IBD model predicts a correlation of genetic distance with geographical distance but not genetic diversity and geographic distance (24). By

contrast, we found (Fig. 3A) that haplotype diversity is strongly correlated with latitude ($R^2 = 0.91$, $P < 0.0001$), with diversity decreasing from south to north, which is consistent with a loss of diversity as populations moved to higher latitudes. In estimating the contribution of SEA and Central-South Asian (CSA) haplotypes to the EA gene pool by haplotype sharing analyses (16), we found that more than 90% of haplotypes in EA populations could be found in SEA and CSA populations, of which about 50% were found in SEA and EA only and 5% found in CSA only (Fig. 3B, see also SOM text). Phylogenetic analysis of private haplotypes indicates greater similarity between EA and SEA populations relative to EA and CSA populations (Fig. 3C). These observations suggest that the geographic source(s) contributing to EA populations were mainly from SEA populations, with rather minor contributions from CSA,

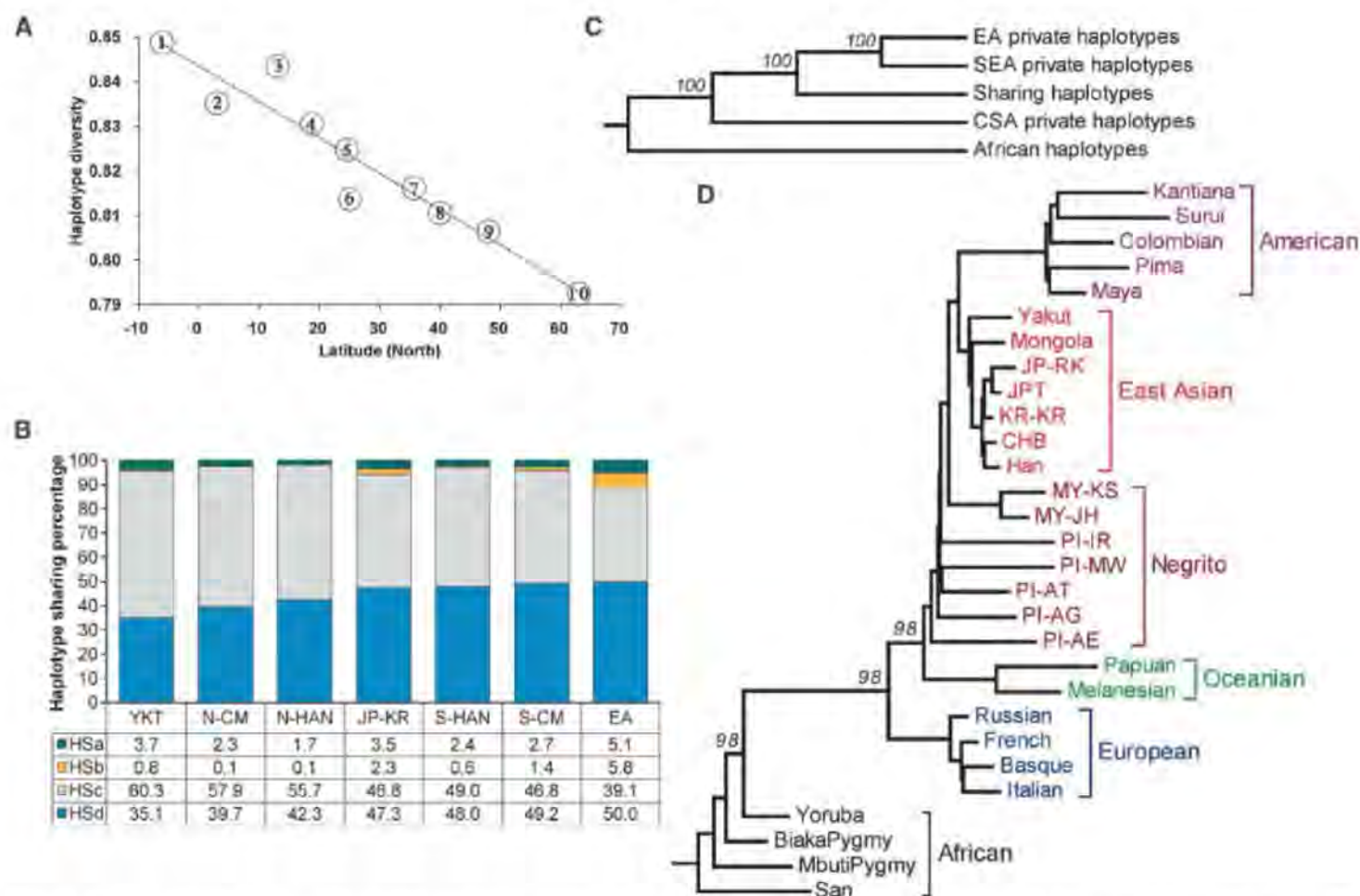


Fig. 3. Analysis of haplotype diversity, haplotype sharing, and population phylogeny. **(A)** Haplotype diversity versus latitudes. Haplotypes were estimated from combined data, and diversity was measured by heterozygosity of haplotypes. HSa, b, c, and d and the corresponding colors show the percentages of EA group haplotypes in each class: HSa, found in CSA only; HSb, found in neither CSA nor SEA; HSc, found in both CSA and SEA; HSD, found in SEA only. Latitudes (y axis) for groups were obtained from the center of sample collection locations. Circled numbers are as follows: 1, Indonesian; 2, Malay; 3, Philippine; 4, Thai; 5, Southern Chinese minorities; 6, Southern Han Chinese; 7, Japanese and Korean; 8, Northern Han Chinese; 9, Northern Chinese minorities; and 10, Yakut. Haplotype heterozygosity of each group was estimated from 100-kb bins and taking together all haplotypes within each group. R^2 for the regression line is 0.91 ($P <$

0.0001). **(B)** Haplotype sharing analysis for EA populations and groups. YKT, Yakut; N-CM, Northern Chinese minorities; N-HAN, Northern Han Chinese; JP-KR, Japanese and Korean; S-HAN, Southern Han Chinese; S-CM, Southern Chinese minorities; EA, East Asian. **(C)** Phylogeny of group private haplotypes. EA private haplotypes: haplotypes found only in EA samples; SEA private haplotypes: haplotypes found only in SEA samples; CSA private haplotypes: haplotypes found only in CSA samples; Shared haplotypes: haplotypes found in all EA, SEA, and CSA samples; African haplotypes were used as outgroup. **(D)** Maximum-likelihood tree of 29 populations. The tree is based on data from 19,934 SNPs. Bootstrap values were based on 100 replicates. Only values on splitting of African and non-African, European and Oceanian and Asian, and Oceanian and Asian are shown.

and that this clinal structure of EA populations arose from prehistoric population divergence rather than IBD or gene flow from CSA populations.

On the basis of increased cultural, linguistic, and genetic diversity, the origins of SEA populations are thought to be more complex than the origins of those to their north. Notably, the Negritos of the Philippines and Malaysia differ from neighboring populations in aspects of their physical appearance, prompting intense speculation about models of human settlement in Southeast Asia. The two-wave hypothesis, which suggests that ancestral Negrito populations settled in Southeast Asia, Australia, and Oceania before a more northerly migration originating in or near the Middle East, and spreading both toward Europe and Northeast Asia via Central Asia (25), has been supported by phylogenetic trees constructed from data on a limited number of protein markers (24, 25). The topology of our population trees, both with and without the data from additional European and Asian populations discussed in (1), is inconsistent with regard to this genetic similarity of European and EA populations (Figs. 1 and 3D). Instead, on the basis of variation at a large number of independent SNPs, we observed that there is substantial genetic proximity of SEA and EA populations (fig. S28). An identical pattern is seen in the population tree of Li *et al.* (1) based on all of their 642,690 SNPs. Our forward-time simulation results under extreme ascertainment scenarios (SOM text) show that the observed phylogeny is not the result of ascertainment bias. Simulation studies also suggest that substantial levels of migration between populations after their initial separation are unlikely to distort the topology of the phylogeny (SOM text).

To unambiguously infer population histories represents a considerable challenge (26). Although this study does not disprove a two-wave model of migration, the evidence from our autosomal data and the accompanying simulation studies (figs. S29 and S30) point toward a history that unites the Negrito and non-Negrito populations of Southeast and East Asia via a single primary wave of entry of humans into the continent.

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S38

Tables S1 to S4

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Positively Selected *G6PD*-Mahidol Mutation Reduces *Plasmodium vivax* Density in Southeast Asians

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency—the most common known enzymopathy—is associated with neonatal jaundice and hemolytic anemia usually after exposure to certain infections, foods, or medications. Although *G6PD*-deficient alleles appear to confer a protective effect against malaria, the link with clinical protection from *Plasmodium* infection remains unclear. We investigated the effect of a common *G6PD* deficiency variant in Southeast Asia—the *G6PD*-Mahidol^{487A} variant—on human survival related to *vivax* and *falciparum* malaria. Our results show that strong and recent positive selection has targeted the Mahidol variant over the past 1500 years. We found that the *G6PD*-Mahidol^{487A} variant reduces *vivax*, but not *falciparum*, parasite density in humans, which indicates that *Plasmodium vivax* has been a driving force behind the strong selective advantage conferred by this mutation.

Malaria is a major cause of human mortality worldwide and is considered to be one of the strongest known forces of evolutionary selection in the recent history of the human genome (1). Host genetic defense mechanisms are likely to have evolved to resist malaria infection in regions where the parasites have been historically prevalent. Among malaria-causing parasites, *Plasmodium falciparum* and *Plasmodium vivax* seem to have exerted strong selective pressure on the cellular phenotype of human erythrocytes, causing increased prevalence of hemoglobinopathies and other inherited blood disorders (1).

Glucose-6-phosphate dehydrogenase (G6PD) is an X-linked essential enzyme that plays a key role in protecting cells from oxidative stress and is particularly important in red blood cells. G6PD deficiency, affecting more than 400 million people worldwide, is associated with several clinical

disorders including neonatal jaundice, hemolytic anemia following infection by certain pathogens, and favism (2). The high overall frequency of *G6PD*-deficient alleles in the population is thought to result from their protective effect against malaria. Evolutionary studies of the *G6PD* locus suggest that local and recent positive selection has targeted the *G6PD*-deficient allele *G6PD*⁴ in Africa (3, 4) and that this process started 2500 to 3800 years before present (YBP) (5). These observations are consistent with signs of recent expansion of *P. falciparum* in Africa (6). However, the clinical link between G6PD deficiency and malaria is less clear. Although a clinical protective effect of G6PD deficiency against human lethal malaria, *P. falciparum*, has been shown in Africa (7), several other reports have not found an association (8, 9).

Most clinical, epidemiological and evolutionary studies of the relation between G6PD deficiency and malaria protection have focused on *falciparum* malaria, particularly in Africa. The role of *G6PD*-deficient alleles in the susceptibility, or resistance, to *vivax* malaria has not been accurately tested and remains anecdotal. Nevertheless, *P. vivax* imposes a considerable burden of disease on the human population and, historically, has been associated with considerable mortality and decreased fertility in human populations (10). Both *P. falciparum* and *P. vivax* coexist in Southeast Asia, with *P. vivax* accounting for over half of malaria cases. Moreover, there is increasing evidence for an ancient origin of *P. vivax* in Asia (11), where its presence apparently predates that of *P. falciparum* (12).

We investigated whether G6PD deficiency increases human survival in Southeast Asia, through its effects on *vivax* and *falciparum* malaria, in an evolutionary and epidemiological study of the Mahidol^{487A} mutation (MIM no. 305900.005). This *G6PD*-deficient variant occurs throughout

greater Southeast Asia, including mainland China, and is most common in Myanmar [Burma] (13). The average allele frequency in Thailand is 12%, but there is distinct local heterogeneity with increased frequency on the western border, particularly in the Mon, Burmese, and Karen populations (13, 14). This mutation is classed as a moderate-to-mild *G6PD* variant with a reduction of 5 to 32% of wild-type activity levels in healthy individuals (13, 15).

We first used an evolutionary approach to detect the potential molecular signature of positive selection at the *G6PD*-Mahidol^{487A} mutation. We genotyped 30 single-nucleotide polymorphisms (SNPs) (including the Mahidol⁴⁸⁷ variant) (table S1) dispersed along a 2.4-Mb region encompassing *G6PD* (Fig. 1A) in a panel of 384 unrelated individuals, the majority of whom are Karen, living in the Suan Phung district of Thailand (16). After reconstructing the phase of extended haplotypes (16), we implemented the long-range haplotype (LRH) test, which identifies alleles that have undergone recent positive selection, i.e., alleles associated with high levels of extended homozygosity at nearby markers and present at high population frequencies (3). The frequency of the Mahidol^{487A} mutation in our population sample was 24% and showed very high levels of extended homozygosity: 63% of Mahidol^{487A}-bearing haplotypes showed complete haplotypic conservation over the entire 2.4-Mb region (Fig. 1, A and B). The high level of homozygosity surrounding Mahidol^{487A} was highly significant given its frequency ($P < 10^{-4}$), when compared with the empirical distribution of allelic homozygosity versus frequency for all X-linked HapMap Phase II SNPs in Han Chinese (17), after matching for SNP density (Fig. 1C). However, the power of the LRH test can be challenged by uncertainties related to phase reconstruction and specific population histories (18). To circumvent this, we compared the observed allelic homozygosity associated with Mahidol^{487A} in males only (whose haplotypic phase is known) with simulations of a ~1-Mb recombining X-linked region for a population experiencing different demographic regimes (16). Consistently, we found a highly significant signal of positive selection at Mahidol^{487A}, independently of the demographic scenario considered ($P < 10^{-4}$ for the constant population size, the bottleneck and the expansion models) (Fig. 1D). The low microsatellite diversity associated with Mahidol^{487A} (Fig. 1E) confirmed the results based on the LRH test. Together, our evolutionary analyses show that the Mahidol^{487A} mutation is under recent and strong positive selection in the Karen population, indicating that this mutation has conferred a strong selective advantage in human survival.

We estimated the age of *G6PD*-Mahidol^{487A} and the selection coefficient that would be consistent with such a strong positive selection. We obtained similar estimates using two different methods (19, 20), which showed that the fre-

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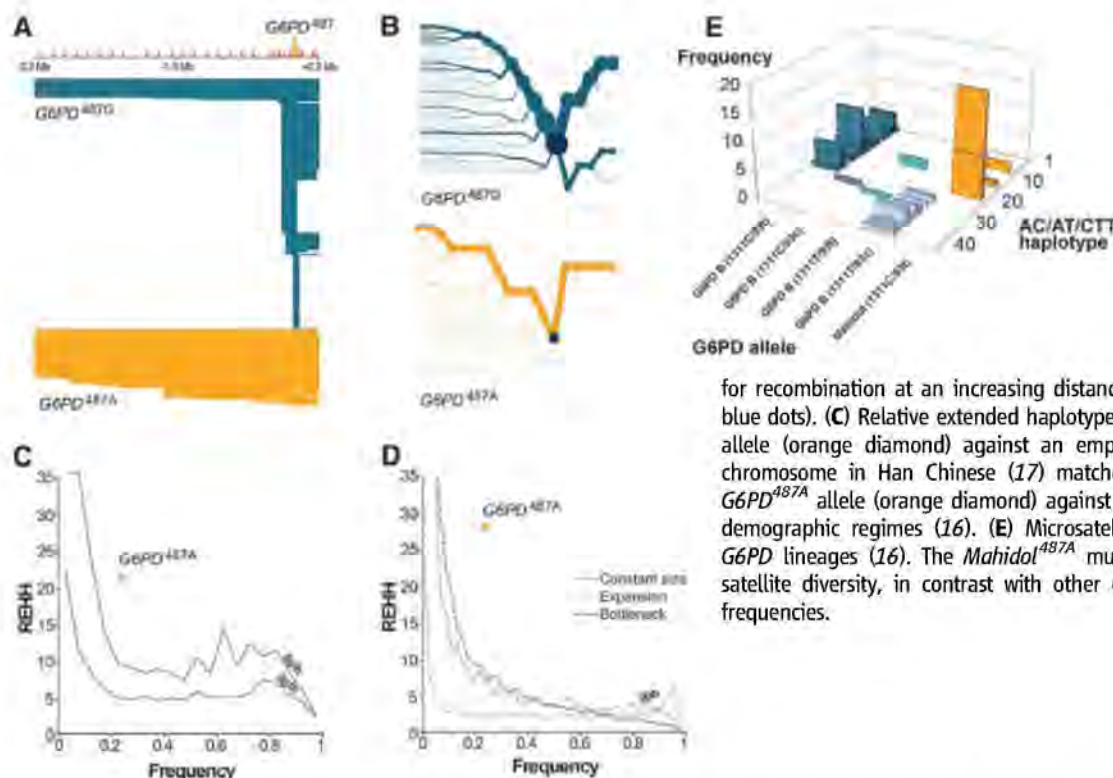


Fig. 1. Positive selection at the *G6PD-Mahidol*^{487A} mutation. (A) Comparison of extent of homozygosity flanking the *G6PD*^{487A} (Mahidol) and the *G6PD*^{487G} (non-Mahidol) alleles over a 2.4-Mb region. Positions of the 30 genotyped SNPs are tagged by red arrows. (B) Haplotype bifurcation plots for the two *G6PD*⁴⁸⁷ alleles. Line thickness represents the number of haplotypes in our sample. Each successive bifurcation corresponds to evidence

for recombination at an increasing distance from the core *G6PD*⁴⁸⁷ alleles (dark blue dots). (C) Relative extended haplotype homozygosity (REHH) of the *G6PD*^{487A} allele (orange diamond) against an empirical distribution of REHH for the X chromosome in Han Chinese (17) matched for SNP density. (D) REHH of the *G6PD*^{487A} allele (orange diamond) against simulated distributions under different demographic regimes (16). (E) Microsatellite diversity associated with different *G6PD* lineages (16). The *Mahidol*^{487A} mutation was associated with low microsatellite diversity, in contrast with other *G6PD* mutations at similar population frequencies.

quency of the *Mahidol*^{487A} mutation started to increase at ~1500 YBP, with a selection intensity of ~0.23 (Table 1). The selection coefficients of *Mahidol*^{487A} are among the strongest detected so far in the human genome, including human immunodeficiency virus (HIV)-protective haplotypes (~0.30) (21), malaria-protective *G6PD*⁴⁸⁷ (~0.2) (5, 20) and β -globin variants (~0.26 and ~0.08) (22, 23), as well as lactase persistence (~0.1) (24).

We investigated the nature of the selective advantage conferred by the *G6PD-Mahidol*^{487A} mutation by testing its influence on the outcome of infection with either *P. falciparum* or *P. vivax*. To this end, we conducted a community-based longitudinal study in the Suan Phung district of Thailand, which has a total population of 5368. From the 3484 participants of the malaria epidemiology study (25), we obtained genotypes at the *G6PD-Mahidol*⁴⁸⁷ position from 925 individuals (16). Between 1998 and 2005, there were 1090 *P. falciparum* clinical episodes in 460 of these individuals, and 524 *P. vivax* clinical episodes in 262 of these individuals. Reliable parasite density data was available for 823 observations of *P. falciparum* parasite density in 400 individuals and for 417 observations of *P. vivax* parasite density in 227 individuals. Details on the sample selection procedures are provided in (16) and summarized in fig. S1.

To test for a genetic association between *G6PD-Mahidol*^{487A} and the number of *Plasmodium* species clinical cases or parasite density, we performed a family-based association test (FBAT), which corrects for spurious association due to population stratification (16). Seventy-seven families were informative for the associa-

Table 1. Joint estimates of the age and the selection coefficient of the *Mahidol*^{487A} mutation. One generation is considered equivalent to 25 years. ML, maximum-likelihood; CI, confidence interval.

Method	Age (generations)	95% CI	Selection coefficient	95% CI
ML deterministic method (19)	60.8	53.7–73.6	0.235	0.20–0.30
Bayesian method (20)	64.4	38–94	0.228	0.16–0.40

tion with the number of *Plasmodium* species clinical cases, 44 of which were informative for the association with *P. falciparum* density and 35 for the association with *P. vivax* density (i.e., some families were infected by different *Plasmodium* species at different times) (16). We found that *Mahidol*^{487A} had no effect on the number of cases of clinical malaria due to either *P. vivax* or *P. falciparum* reported for each individual during the 7-year observation period (16). This is likely due to the high heterogeneity of exposure to infection in this area of low transmission intensity, where virtually all infections by either parasite species lead to symptomatic episodes (25). Our analyses also revealed that *Mahidol*^{487A} was not significantly associated with *P. falciparum* density, using both annual values of parasite density (16) or mean density values across all years under any genetic model (table S2). By contrast, *Mahidol*^{487A} was significantly associated with reduced *P. vivax* density using both annual values of parasite density (χ^2 test, $P = 0.029$) and mean density values across years [χ^2 test; dominant model $P = 0.011$, additive model $P = 0.016$, recessive model $P = 0.048$ (table S2)] taking into account age and environmental covariates that affect parasite density (16, 26). A permutation test with 100,000 iterations confirmed that

these results were significant (χ^2 test, $P = 0.017$).

To verify this association, we performed a population-based association study between *Mahidol*^{487A} and *Plasmodium* parasite density in the whole population, excluding all individuals used in the FBAT analyses (16). *Mahidol*^{487A} significantly reduced *P. vivax* parasite density (the most significant P value was obtained for the dominant model, χ^2 test, $P = 0.006$), whereas no association was observed with *P. falciparum* parasite density for any genetic model (table S2). When considering both the family-based and the population-based data sets, *P. vivax* parasite density decreased with age (χ^2 test, $P < 0.001$), indicative of the acquisition of antiparasite immunity (Fig. 2A). Mean *P. vivax* density was reduced by 30% in females heterozygous for the *Mahidol*⁴⁸⁷ mutation (non-Mah/Mah) and 61% in females homozygous for *Mahidol*^{487A} (Mah/Mah) compared with non-Mah/non-Mah females; parasite density was reduced by 40% in hemizygous males for *Mahidol*^{487A} (Mah/Y) compared with non-Mah/Y males (Fig. 2B). *Mahidol*^{487A} accounted for 3.3% of the observed variation in *P. vivax* density. Although increasing age was again associated with decreasing *P. falciparum* density (χ^2 test, $P < 0.001$) (Fig. 2C), there was

no significant effect of *Mahidol*^{487A} on *falciparum* density (Fig. 2D).

A *Mahidol*^{487A} gene dose effect on *P. vivax* density was observed, whereby parasite density tends to be lower in *Mah/Mah* females with respect to both non-*Mah/Mah* females and *Mah/Y* males (Fig. 2B). Two main factors can explain the observed tendency. First, G6PD activity in heterozygous non-*Mah/Mah* females depends on the random inactivation of the X-chromosome: Each heterozygous female will harbor two populations of red blood cells (expressing *Mahidol*^{487A} or *Mahidol*^{487G}), and their relative abundance is unpredictable. Indeed, when measuring G6PD activity in Karen individuals using the fluorescence spot test (16), we ob-

served that the non-*Mah/Mah* genotype can express either normal or deficient phenotypes (table S3). Second, the *Mahidol*^{487A} mutation markedly increases protein thermal instability with consequences for enzyme activity (15). Hence, in *P. vivax* infection, which induces high fever, G6PD deficiency may be more pronounced than in noninfected individuals, which contributes to the reduction in parasite density. Because genotype-phenotype correlations for G6PD can be complex, we analyzed our data using G6PD phenotypes rather than genotypes. We confirmed that both age and G6PD-deficient phenotype significantly decreased *P. vivax* density (χ^2 test, $P < 0.001$ and $P = 0.039$, respectively) (16).

Although the precise mechanism underlying this protective effect is unknown, it is likely to be related to the effects of G6PD deficiency on red cell physiology, particularly by increasing oxidative stress. Young red cells (i.e., reticulocytes) contain more antioxidant enzymes than mature red cell populations (27). The preference shown by *P. vivax* for reticulocytes suggests that *P. vivax* is more sensitive to oxidative stress than *P. falciparum*, which is known to have no red-cell preference (28). Under these conditions, reduced G6PD activity would have a greater effect on *P. vivax*.

The historical expansion of malaria has been linked to that of agriculture, which generated breeding grounds for mosquitoes and increased human population density (29), thereby facilitating human-mosquito contact and the conditions for stable malaria transmission. In East Asia, farming is mainly associated with the development of rice culture in China ~8000 YBP. Although there is evidence of rice cultivation in Southeast Asia dating back to 4200 YBP, it developed mainly over the last 2000 years (30). The Karen people, belonging to the Sino-Tibetan language group, are thought to descend from Tibetan people who entered Myanmar ~1500 YBP (31). It is noteworthy that the estimated age of *Mahidol*^{487A}, at ~1500 YBP, coincides with the proposed arrival of the Karens into the region and with the time at which rice started to be extensively cultured. This supports a link between the selective advantage conferred by the *G6PD-Mahidol*^{487A} mutation and its protective effect against *vivax* malaria.

In conclusion, we showed that the *G6PD-Mahidol*^{487A} mutation has been under strong positive selection for the last 1500 years and that it reduces *P. vivax* parasite density in humans. These findings provide evidence that *vivax* malaria has been a driving force behind the selective advantage conferred by the *Mahidol*^{487A} mutation and supports the notion that *P. vivax* historically had a considerable impact on human health (10), at least in Southeast Asia. Indeed, *P. vivax* infection is not only responsible for clinical malarial attacks, but has also been implicated in causing low birth weight (32) and malnutrition in children (33), both of which have strong impacts on childhood survival. The significant health burden imposed by *P. vivax* has been seriously underestimated, and increased global efforts to combat malaria should encompass *P. vivax* as well as *P. falciparum* malaria.

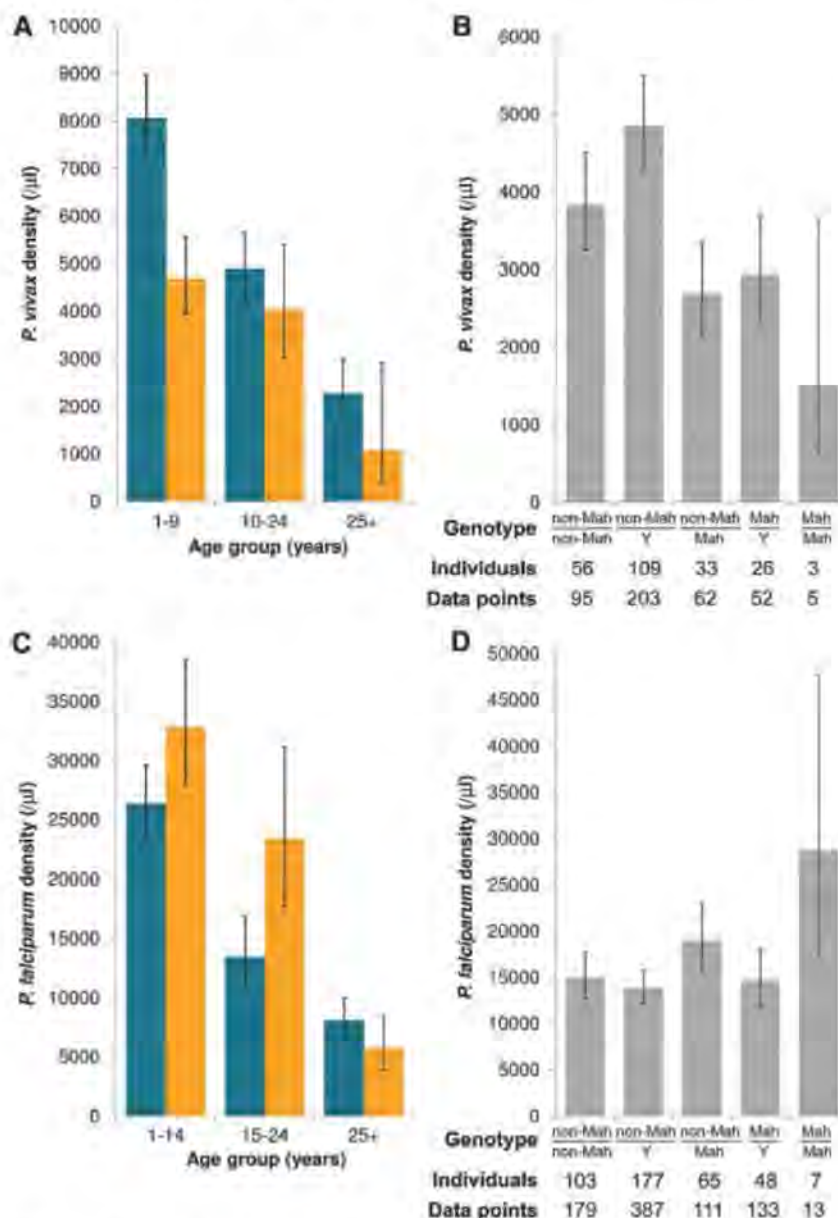


Fig. 2. Effect of *G6PD* status on parasite densities. (A) *P. vivax* and (C) *P. falciparum* parasite densities (means $\pm$ SD) according to age group and *G6PD-Mahidol*⁴⁸⁷ status (blue, non-*Mahidol*; orange, *Mahidol*). (B) *P. vivax* and (D) *P. falciparum* parasite densities (means $\pm$ SD) according to *G6PD-Mahidol*⁴⁸⁷ genotype status taking into account age (16). Data points refer to the total number of observations of parasite densities obtained for each group of individuals. Note that all data were used, including the FBAT informative families.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5959/1546/DC1
Materials and Methods

Fig. S1

Tables S1 to S3

References

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MicroRNA-206 Delays ALS Progression and Promotes Regeneration of Neuromuscular Synapses in Mice

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Amiotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by loss of motor neurons, denervation of target muscles, muscle atrophy, and paralysis. Understanding ALS pathogenesis may require a fuller understanding of the bidirectional signaling between motor neurons and skeletal muscle fibers at neuromuscular synapses. Here, we show that a key regulator of this signaling is miR-206, a skeletal muscle-specific microRNA that is dramatically induced in a mouse model of ALS. Mice that are genetically deficient in miR-206 form normal neuromuscular synapses during development, but deficiency of miR-206 in the ALS mouse model accelerates disease progression. miR-206 is required for efficient regeneration of neuromuscular synapses after acute nerve injury, which probably accounts for its salutary effects in ALS. miR-206 mediates these effects at least in part through histone deacetylase 4 and fibroblast growth factor signaling pathways. Thus, miR-206 slows ALS progression by sensing motor neuron injury and promoting the compensatory regeneration of neuromuscular synapses.

Amyotrophic lateral sclerosis (ALS) is the most common adult motor neuron disease (1). Symptoms of the disease include atrophy and paralysis of lower limb and respiratory muscles because of the degeneration of motor neurons. There is currently no effective treatment. Thus, identification of the signaling pathways and cellular mediators of ALS remains a major challenge in the search for novel therapeutics (2).

In light of recent studies implicating microRNAs (miRNAs) in stress responses in muscle (3), we investigated whether disease progression in a mouse model of ALS was accompanied by changes in expression of miRNAs. We compared miRNA expression in skeletal muscles from the lower limbs of normal adult mice and G93A-SOD1 transgenic mice (4, 5) that express a low copy number of a mutant form of superoxide dismutase (SOD1) in which glycine-93 is replaced with alanine (G93A-SOD1), as seen in a subset of human ALS patients. These mice recapitulate the progression of human ALS symptoms (4, 5). Of 320 miRNAs tested, the muscle-specific miRNA miR-206 (6, 7) was the most dramatically up-regulated in G93A-SOD1 muscles (Fig. 1A and fig. S1, A and B) (8). Up-regulation of miR-206 coincided with the onset of neurological symptoms, as indicated by levels of miR-206 in healthy G93A-SOD1 trans-

genic mice being similar to those in wild-type littermates (Fig. 1A and fig. S1B).

Because ALS leads to denervation of skeletal muscle (1), we determined whether miR-206 up-regulation was a consequence of denervation. Indeed, 10 days after severing the sciatic nerve of wild-type mice to denervate lower leg muscles, levels of mature and primary miR-206 (pri-miR-206) transcripts were robustly increased in three muscles that contain predominantly fast-twitch fibers, extensor digitorum longus (EDL), tibialis anterior (TA), and gastrocnemius/plantaris (G/P) (Fig. 1B and fig. S2) (9). miR-206 levels were higher in normally innervated soleus, which contains predominantly slow myofibers, and up-regulation after denervation was correspondingly less substantial (fig. S2).

miR-206 is a skeletal muscle-specific miRNA in humans and mice that is generated from a bicistronic transcript that also encodes miR-133b (fig. S3, A and B) (7, 10). Two other homologous miRNA pairs, miR-1-1/133a-2 and miR-1-2/133a-1, are encoded on separate chromosomes and are expressed in skeletal and cardiac muscle (6, 10). Consistent with its transcription from the same promoter, miR-133b was also up-regulated after denervation, whereas miR-1 and miR-133a were down-regulated (Fig. 1B and fig. S2).

Previous studies have implicated the myogenic basic helix-loop-helix (bHLH) proteins MyoD and myogenin in denervation-dependent gene expression (fig. S4A) (11). Three evolutionarily conserved E-boxes (CANNTG), which are binding sites for MyoD and myogenin, are located between -910 and -765 base pairs (bp) upstream of the start of the precursor (pre-miR-206) stem loop, within a genomic region that was previously shown to be enriched for MyoD binding by using chromatin from muscle cells (Fig. 1C and fig. S4B) (7). Heterodimers of MyoD and its bHLH partner E12 bind these sites (fig. S4C). In cultured cells, MyoD activated the

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Fig. 1. Regulation of miR-206 in response to ALS and denervation. **(A)** Up-regulation of miR-206 during the progression of ALS in G93A-SOD1 mice as determined by means of Northern blot and quantified by densitometry. $*P < 0.0001$, Student's t test; $n = 2$ to 4 mice. **(B)** Transcripts of miR-206, miR-133b, miR-1, and miR-133a were detected by means of real-time PCR in TA muscles after 10 days of denervation (+). The contralateral muscle was used as a control (-). $*P < 0.02$, $**P < 0.005$, Student's t test; $n = 3$ to 4 mice. **(C)** Sequence alignment of the mouse *miR-206* 5' flanking sequence from different species shows the conserved upstream region containing E-boxes. Position (0) denotes the start of pre-miR-206. Bracketed region represents the identified enhancer region. **(D)** β -galactosidase staining of G/P muscle isolated from denervated transgenic mice containing a lacZ transgene controlled by the wild-type (WT) enhancer or the mutant enhancer (containing mutated E-boxes) [(C), bracket]. Contralateral muscle was used as a control. Lower panels show transverse section of muscle. Scale bar, 200 μ m. Values represent mean $\pm$ SEM.

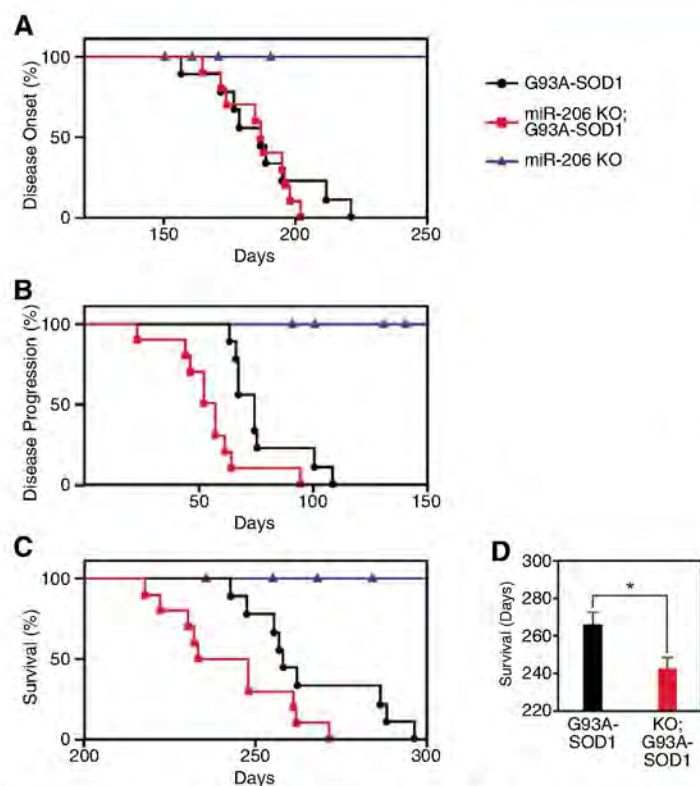
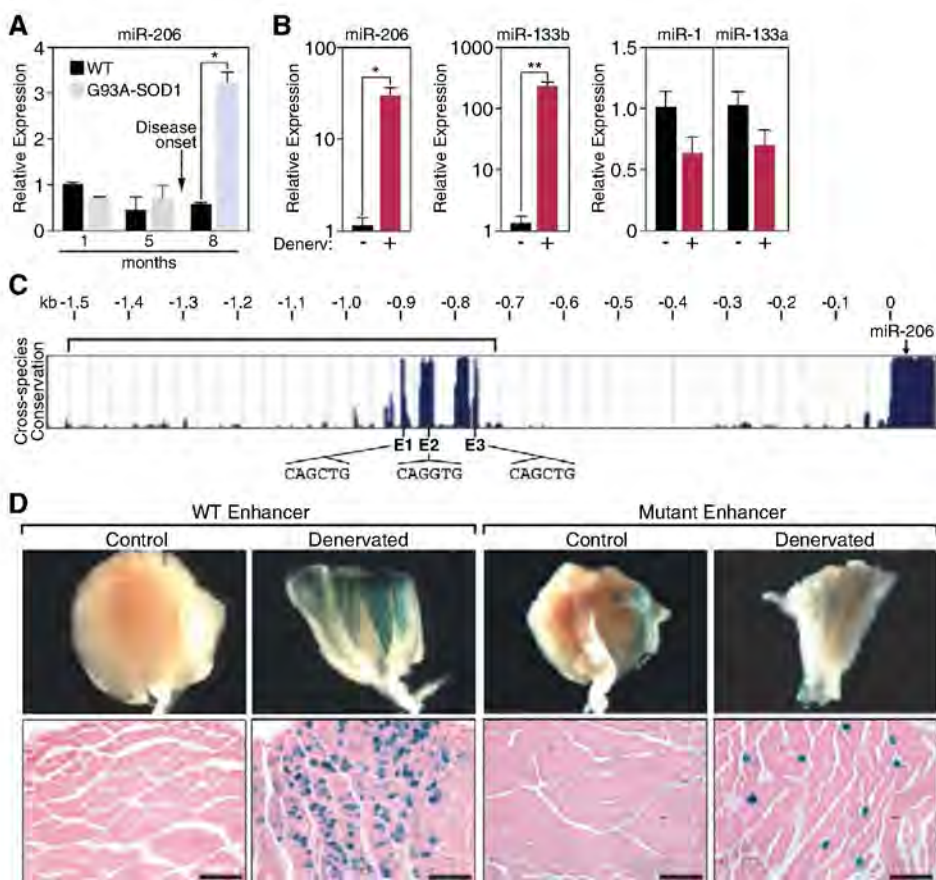
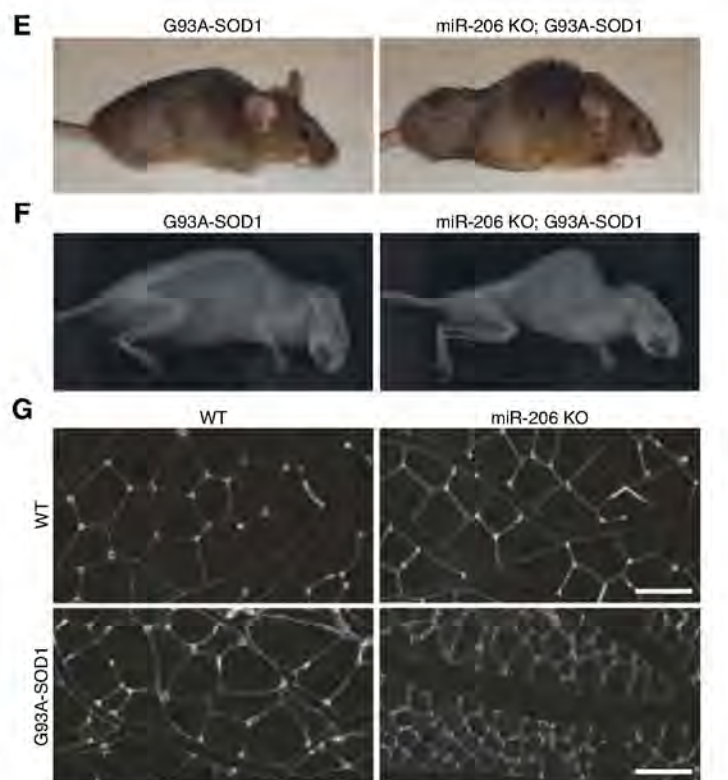


Fig. 2. Regulation of ALS pathogenesis by miR-206. **(A)** Age of disease onset for G93A-SOD1 (black) ($n = 9$ mice) (mean, 188 days), miR-206^{-/-}; G93A-SOD1 (red) ($n = 10$ mice) (mean, 187 days), and miR-206 KO (blue) littermates. **(B)** Days of disease progression for G93A-SOD1 (mean, 78 days) and miR-206^{-/-}; G93A-SOD1 littermates (mean, 56 days). $P < 0.005$, log-rank test. **(C)** Survival curve for G93A-SOD1 (mean, 266 days), miR-206^{-/-}; G93A-SOD1 (mean, 244 days). $P < 0.05$, log-rank test. **(D)** Survival of G93A-SOD1



and miR-206^{-/-}; G93A-SOD1 mice. $*P < 0.02$, Student's t test. **(E)** G93A-SOD1 and miR-206^{-/-}; G93A-SOD1 mice at approximately 7.5 months of age. There is severe atrophy of hindlimb muscles in miR-206^{-/-}; G93A-SOD1 mice. **(F)** X-ray reveals kyphosis in miR-206^{-/-}; G93A-SOD1 mice. **(G)** Wheat-germ agglutinin (WGA) staining of transverse sections of muscle show accelerated muscle atrophy in miR-206^{-/-}; G93A-SOD1 mice as compared with that of G93A-SOD1 littermates. Scale bar, 100 μ m. Values represent mean $\pm$ SEM.

expression of a reporter controlled by this region, and mutations of the E-boxes abolished the responsiveness to MyoD (fig. S4D).

In transgenic mice harboring an *Escherichia coli* β -galactosidase (*lacZ*) reporter controlled by the *miR-206* 5' regulatory region, *lacZ* expression was dramatically up-regulated in muscle after

surgical denervation (Fig. 1D). Mutations in the three E-boxes reduced responsiveness of the *miR-206* enhancer to the minimal level of the *hsp68* basal promoter (Fig. 1D and fig. S4E). Thus, direct regulation by myogenic bHLH factors leads to transcriptional activation of *miR-206* in response to skeletal muscle denervation.

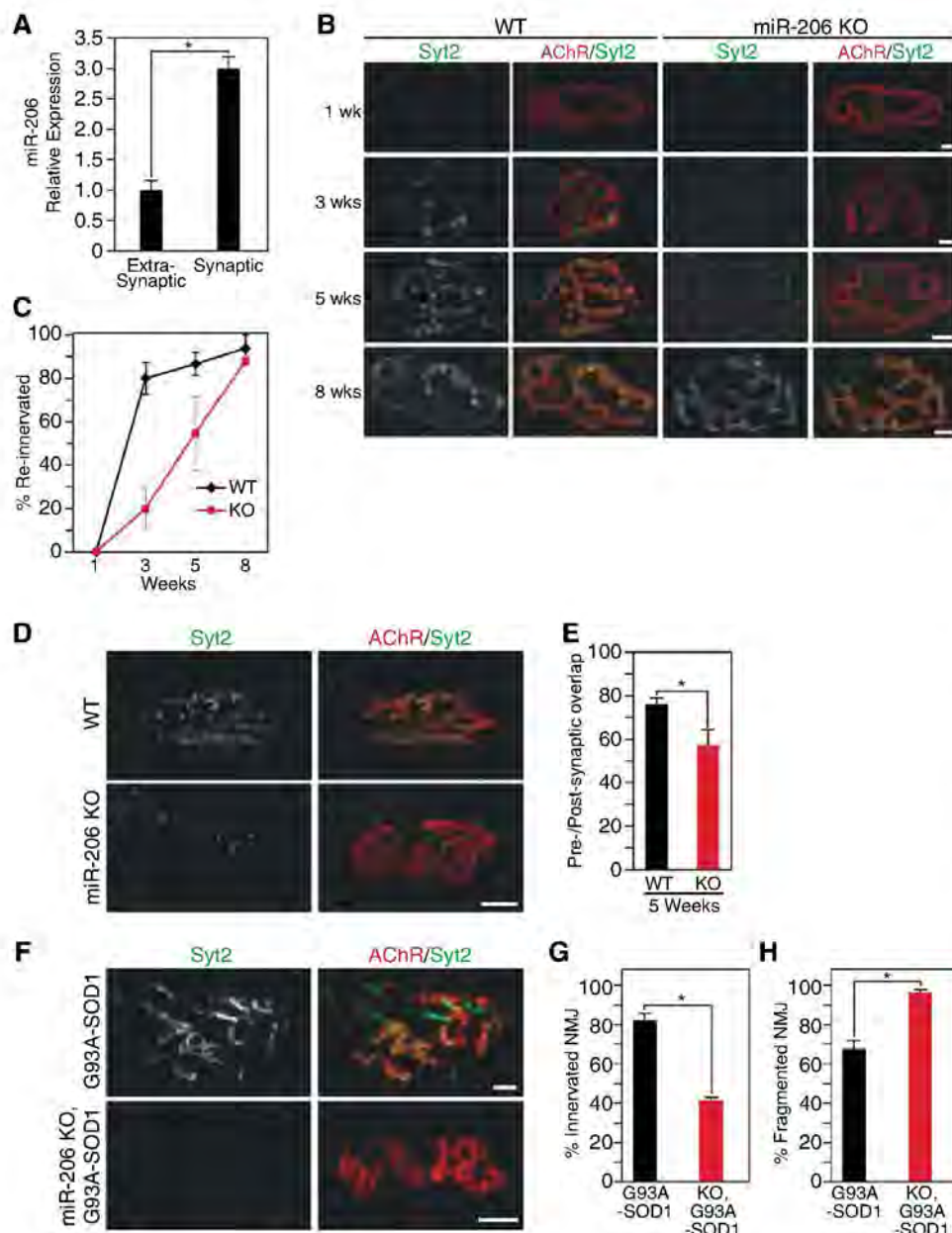


Fig. 3. Delayed NMJ reinnervation in *miR-206* mutant mice. **(A)** Quantitative real-time PCR reveals *miR-206* expression is enriched in synaptic regions of muscle fibers. $*P < 0.0002$, Student's *t* test; $n = 3$ mice. **(B)** After sciatic nerve transection (as indicated in weeks), a delay in reinnervation is seen in *miR-206*^{-/-} mice (KO) as compared with WT mice as detected by the superimposition of synaptotagmin 2 (Syt 2) staining (green) with BTX (red). Scale bar, 10 μ m. **(C)** Time course and quantification of the number of reinnervated NMJs in WT and *miR-206*^{-/-} (KO) mice after sciatic nerve transection. $n = 2$ to 6 mice. **(D)** Immunohistochemistry shows a delay in presynaptic differentiation and partial reoccupancy of postsynaptic sites in *miR-206*^{-/-} NMJs 5 weeks after sciatic nerve transection. Scale bar, 10 μ m. **(E)** Postsynaptic area occupied by the reinnervating nerve (in percent) 5 weeks after cutting the sciatic nerve in WT and *miR-206*^{-/-} (KO) mice. $*P < 0.02$, Student's *t* test. **(F)** Immunohistochemistry shows increased NMJ dysfunction and denervation in *miR-206*^{-/-};G93A-SOD1 as compared with G93A-SOD1 littermates. Scale bar, 10 μ m. **(G)** Number of innervated NMJs in G93A-SOD1 and *miR-206*^{-/-};G93A-SOD1 mice at 7 months of age. $*P < 0.0005$, Student's *t* test. **(H)** Number of fragmented NMJs in G93A-SOD1 and *miR-206*^{-/-};G93A-SOD1 mice at 7 months of age. $*P < 0.005$, Student's *t* test. Values represent mean $\pm$ SEM.

The increased level of *miR-206* in ALS might be an innocuous correlate, a contributor to pathology, or part of an ultimately inadequate compensatory effort. To distinguish these possibilities, we generated targeted mutants in which *miR-206* expression was abolished without affecting *miR-133b* expression (fig. S5, A to D). Mice that were homozygous for the targeted deletion of *miR-206* showed no obvious abnormalities in weight, behavior, the architecture and fiber-type distribution of skeletal muscles, or expression of pri-*miR-133b* and pri-*miR-1* (fig. S5, E and F).

Next, we generated *miR-206*^{-/-} mice that express a low copy number of G93A-SOD1. Loss of *miR-206* did not affect disease onset but did accelerate disease progression and diminish survival (Fig. 2, A to D). The exacerbation of disease symptoms in *miR-206*^{-/-} mice was accompanied by an accelerated atrophy of skeletal muscle, leading to kyphosis, paralysis, and death (Fig. 2, E to G, and movies S1 and S2). *miR-206*^{-/-} mice lacking the G93A-SOD1 transgene showed no overt phenotype or decrease in survival up to 300 days (Fig. 2C). Thus, increased *miR-206* expression in response to denervation counteracts, albeit ultimately unsuccessfully, the pathogenesis of ALS.

How does *miR-206* act to extend survival in ALS? In that motor neuron pathology plays a key role in ALS, whereas *miR-206* is expressed exclusively in muscles, we suspected that the *miRNA* affects nerve-muscle interactions. Indeed, a transcript derived from the *miR-206/133b* locus was originally identified as a synapse-associated noncoding RNA called 7H4 (12), as has been seen for other genes encoding components of the post-synaptic apparatus (13). Although the reported 7H4 sequence did not include *miR-206*, reverse transcription polymerase chain reaction (RT-PCR) demonstrated that *miR-206* sequences are in fact included in this synapse-enriched transcript (Fig. 3A and fig. S6, A and B) (12). This expression pattern focused our attention on the neuromuscular junction (NMJ).

We examined the architecture of NMJs in neonatal and adult wild-type and *miR-206*^{-/-} mice using labels for the postsynaptic membrane [α -bungarotoxin (BTX)], motor axons (antibodies to neurofilaments), and nerve terminals (antibodies to the synaptic vesicle protein synaptotagmin 2) (14). NMJs of embryonic, neonatal, and adult mutant mice showed no obvious differences when compared with age-matched wild-type NMJs (fig. S7, A and B). Thus, *miR-206* is dispensable for formation and maturation of the NMJ.

In contrast, *miR-206* profoundly influenced formation of new NMJs after nerve injury, which denervates muscle. Three weeks after surgical denervation, both wild-type and *miR-206*^{-/-} mice exhibited similar degrees of muscle atrophy (fig. S8, A and B), but reinnervation of denervated muscles by motor axons was delayed in the absence of *miR-206*. Regenerating axons preferentially reinnervate original synaptic sites after denervation (15, 16), so we quantified the

number of postsynaptic sites apposed by nerve. Because postsynaptic acetylcholine receptor (AChR) aggregates remain largely intact after denervation (17), reinnervation can be accurately assessed by the superimposition of BTX (red) and synaptotagmin (green) staining. In wild-type mice, reinnervation began between 2 and 3 weeks after nerve cut and was nearly complete by 5 weeks after injury (Fig. 3, B and C, and fig. S9A).

In contrast, reinnervation of *miR-206*^{-/-} TA muscles did not begin until 3 weeks after injury and remained retarded for at least 2 more weeks (Fig. 3, B and C, and fig. S9A). Reinnervation was also delayed when the nerve was crushed rather than cut; in this procedure, no gap is generated, and regeneration to targets occurs more rapidly and reliably than after nerve cut (fig. S9, B to E).

To rule out defects in axonal regeneration, we visualized the nerves near the muscle entry point at 3 weeks after transection and found similar numbers of nerve fibers in wild-type and *miR-206*^{-/-} nerves, indicating that axonal growth was unimpaired in the mutants (fig. S9F). Thus, the prolonged delay in reinnervation in the absence of *miR-206* may result from the lack of a local signal emanating from muscle that influences

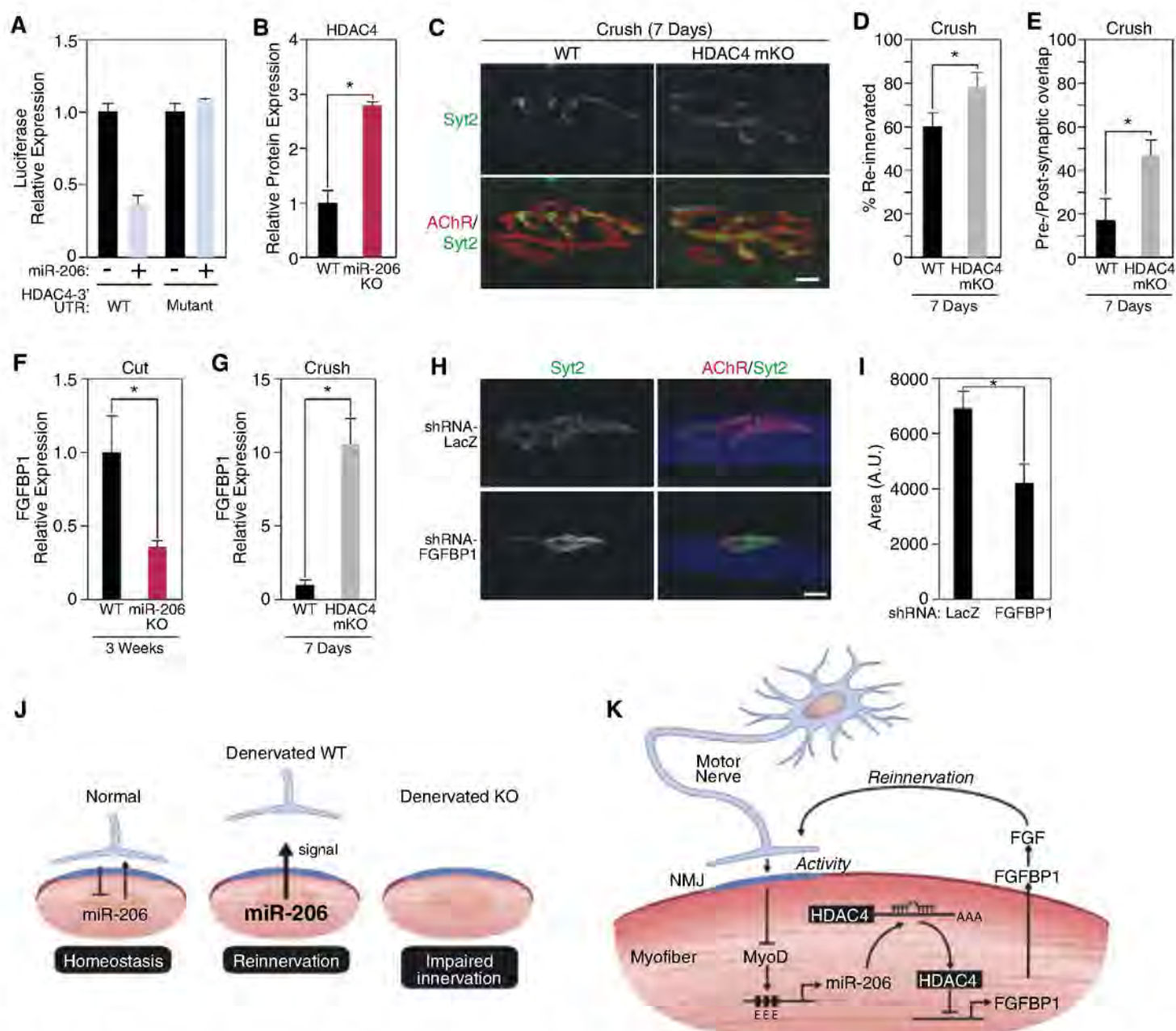


Fig. 4. MiR-206 regulates reinnervation through HDAC4 and FGFBP1. **(A)** Luciferase activity of COS1 cells cotransfected with WT or mutant HDAC4 3' untranslated region (3'UTR)-luciferase constructs with a miR-206 expression plasmid. **(B)** Quantitation of HDAC4 protein expression in muscle lysates isolated from WT and *miR-206*^{-/-} (*miR-206* KO) mice after 3 weeks of denervation. $*P < 0.02$, Student's *t* test; $n = 3$ mice. **(C)** Immunohistochemistry shows an increase in reinnervation in HDAC4 mKO mutant mice as compared with that of WT mice 7 days after nerve crush. Scale bar, 10 μ m. **(D)** Number of reinnervated NMJs in WT and HDAC4 mKO mice after sciatic nerve crush for 7 days. $*P < 0.05$, Student's *t* test; $n = 3$ to 8 mice. **(E)** Postsynaptic area occupied by the reinnervating nerve (in percent) 7 days after crushing the sciatic nerve in

WT and HDAC4 mKO mice. $*P < 0.02$. **(F)** Decrease in expression of *Fgfbp1* transcripts in *miR-206*^{-/-} (KO) muscles 3 weeks after nerve transection. $*P < 0.02$, Student's *t* test; $n = 3$ to 5 mice. **(G)** Increase in expression of *Fgfbp1* transcripts in HDAC4 mKO muscles 7 days after nerve crush. $*P < 0.0005$, Student's *t* test; $n = 3$ to 5 mice. **(H)** Immunohistochemistry shows an inhibition of synaptic-vesicle clustering in neonatal NMJs upon knockdown of FGFBP1. Scale bar, 10 μ m. **(I)** NMJ size in muscle fibers expressing LacZ or FGFBP1 shRNAs. Mice were electroporated at 0 days after birth (P0) and analyzed at P8. $*P < 0.02$, Student's *t* test; $n = 4$ mice. Values represent mean $\pm$ SEM. **(J)** Schematic of miR-206 up-regulation and function after denervation. **(K)** Proposed mechanism of miR-206-dependent reinnervation.

interaction of regenerated motor axons with muscle fibers. Consistent with this conclusion, many mutant synaptic sites were only partially reoccupied by the regenerated nerve (Fig. 3, D and E, and fig. S9E). Moreover, synaptic vesicles failed to aggregate properly in regenerated mutant nerve terminals, and motor axons often sprouted beyond miR-206^{-/-} NMJs, suggesting a possible lack of “stop and differentiate” signals emanating from the muscle (Fig. 3, D and E, and fig. S9G). Similar defects have been documented in mutant mice that lack muscle-derived organizers of presynaptic differentiation and maturation (14), suggesting decreased levels of muscle-derived factors that promote reinnervation once axons approach muscle fibers.

The role of miR-206 in reinnervation after nerve damage may explain its salutary function in ALS. As motor neurons die in ALS, denervated muscle fibers are reinnervated by the axon branches of the surviving motor neurons. Compensatory reinnervation may account for the clinical observations that ALS is nearly asymptomatic in humans until a large fraction of motor neurons have died, at which point the few remaining ones cannot sufficiently compensate (18, 19). Consistent with this idea, NMJs were remarkably similar in miR-206^{-/-};G93A-SOD1 and G93A-SOD1 as compared with that of wild-type mice before the onset of ALS symptoms (fig. S10). Subsequently, miR-206^{-/-};G93A-SOD1 NMJs were disorganized and showed imperfect colocalization of nerve and postsynaptic sites (Fig. 3, F to H). In that a majority of NMJs in G93A-SOD1 mice at this stage are products of reinnervation (18), we hypothesize that the ability of miR-206 to promote reinnervation probably slows disease progression in ALS.

How might miR-206 promote a partially successful compensatory response to denervation? Histone deacetylase 4 (HDAC4) mRNA is among the strongest computationally predicted targets of miR-206 (fig. S11A) (6, 20). HDAC4 has been implicated in the control of neuromuscular gene expression (21, 22), and the closely related miR-1 inhibits translation of HDAC4 mRNA in vitro (6). Using reporter constructs, we showed that miR-206 represses HDAC4 translation (Fig. 4A). Moreover, HDAC4 protein expression was increased in skeletal muscle of miR-206^{-/-} animals as compared with that of wild-type controls after denervation (Fig. 4B and fig. S11B). *Hdac4* mRNA levels were not changed in miR-206^{-/-} mice, indicating that miR-206 acts in this instance by translational inhibition rather than by mRNA destabilization (fig. S11C) (23). Previous work demonstrated that HDAC4 induces *myogenin* expression through the repression of *Dach2* expression, a repressor of *myogenin* (21, 22). As expected, *Dach2* transcripts were decreased and *myogenin* transcripts were increased in miR-206^{-/-} mice (fig. S11, D to F).

To determine whether HDAC4 acts in a manner antagonistic to miR-206, as predicted for a miRNA target, we generated mice in which

Hdac4 was selectively deleted in skeletal muscle (HDAC4 mKO). NMJs formed and matured normally in the absence of HDAC4 (fig. S12A), but mutant muscles were reinnervated more rapidly than those of controls after nerve crush or cut (Fig. 4, C to E, and fig. S12, B to D), a phenotype opposite that of miR-206^{-/-} mice. These findings suggest that miR-206 functions to counteract the negative influence of HDAC4 on reinnervation after injury.

The phenotypes of miR-206 and HDAC4 mutant mice suggested that miR-206 and HDAC4 have opposing effects on retrograde signals required for reinnervation. We therefore searched for muscle-derived synaptic organizing factors that were affected in opposite ways by miR-206 and HDAC4. The mRNA levels of several known regulators of synapse formation (14, 24, 25), including fibroblast growth factor 7 (FGF-7), FGF-10, and FGF-22, were not changed between miR-206^{-/-} and wild-type mice (fig. S13). However, an FGF binding protein, FGFBP1, was down-regulated in muscles of miR-206^{-/-} mice and up-regulated in muscles of HDAC4 mKO mice after denervation (Fig. 4, F and G). FGFBP1 is a secreted factor that interacts with FGF-7, FGF-10, and FGF-22 family members and potentiates the bioactivity of FGF-7 in rat L6 myoblasts by releasing sequestered FGF from the extracellular matrix (26). Because FGF-7, FGF-10, and FGF-22 are muscle-derived regulators that promote presynaptic differentiation at the NMJ (14), we hypothesized that FGFBP1 could potentiate the effects of FGFs during reinnervation. Consistent with this idea, recombinant FGFBP1 enhanced the ability of FGF10 to promote differentiation of vesicle-rich varicosities in cultured motor neurons (fig. S14, A and B).

To probe the role of FGFBP1 in vivo, we used an interfering RNA (fig. S15). Knockdown of FGFBP1 in vivo inhibited the maturation of neonatal NMJs: AChR clusters were smaller than those in control myofibers, and presynaptic vesicle clustering was perturbed (Fig. 4, H and I). These defects resembled those documented above in miR-206^{-/-} mice after denervation, suggesting that miR-206 and HDAC4 promote and impede NMJ innervation, respectively, via opposing effects on FGFBP1.

It has long been known that denervated muscle is readily reinnervated, whereas innervated muscle cannot be hyperinnervated (27, 28), suggesting that muscle fibers can sense whether or not they are innervated and respond to denervation by enhancing their susceptibility to reinnervation. Our results indicate that miR-206 regulates one important pathway involved in this bidirectional signaling (Fig. 4, J and K).

Our results reveal miR-206 as a modifier of ALS pathogenesis and suggest that the salutary actions of miR-206 are mediated by muscle-derived factors that promote nerve-muscle interactions in response to motor neuron injury. MiR-206 expression is highly enriched in slow muscles, which are resistant to denervation in mouse

models of ALS (29). Perhaps increased miR-206-dependent retrograde signaling helps protect slow myofibers. Recent studies have identified mutations in humans with ALS in the genes encoding TDP-43 and FUS, which regulate various aspects of RNA metabolism (30–32) and biochemically interact with the miRNA processing enzyme Drosha (33, 34). Moreover, the related *Caenorhabditis elegans* miRNA, miR-1, also regulates nerve terminal function and retrograde signaling (35). Together with these results, the identification of miR-206 as a modifier of ALS pathogenesis provides a new perspective on the mechanisms underlying this disease and suggests opportunities for intervention through the modulation of miR-206 or the downstream pathways it regulates.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5959/1549/DC1
Materials and Methods
Figs. S1 to S15
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Movies S1 and S2

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Norbin Is an Endogenous Regulator of Metabotropic Glutamate Receptor 5 Signaling

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Metabotropic glutamate receptor 5 (mGluR5) is highly expressed in the mammalian central nervous system (CNS). It is involved in multiple physiological functions and is a target for treatment of various CNS disorders, including schizophrenia. We report that Norbin, a neuron-specific protein, physically interacts with mGluR5 *in vivo*, increases the cell surface localization of the receptor, and positively regulates mGluR5 signaling. Genetic deletion of Norbin attenuates mGluR5-dependent stable changes in synaptic function measured as long-term depression or long-term potentiation of synaptic transmission in the hippocampus. As with mGluR5 knockout mice or mice treated with mGluR5-selective antagonists, Norbin knockout mice showed a behavioral phenotype associated with a rodent model of schizophrenia, as indexed by alterations both in sensorimotor gating and psychotomimetic-induced locomotor activity.

In view of the numerous important roles of mGluR5 in the CNS (1–5), we searched for endogenous regulators of this receptor. We used the carboxy-terminal domain of mGluR5a (Ala⁸²⁶ to Leu¹¹⁷¹) as bait in a yeast two-hybrid screen. Sixteen interacting clones (6) were isolated, including several known mGluR5-interacting proteins, such as Homer1 (7) and calmodulin (8). Three clones encoded the C terminus of Norbin (Fig. 1A, top, and fig. S1A) (9). Norbin, also known as Neurochondrin (10), is a 75-kD neuronal protein without any known functional domain (11). When tested with all known mGluR receptors (mGluR1 to mGluR8) (12), Norbin (Glu⁴⁹⁹ to Pro⁷²⁹) specifically interacted with a subset of group I mGluRs, namely mGluR1a, mGluR5a, and mGluR5b (Fig. 1A, bottom).

Direct interaction of Norbin and mGluR5 was confirmed by GST (glutathione *S*-transferase) pull-down (fig. S1B) and coimmunoprecipitation

experiments (fig. S1C). Endogenous Norbin and mGluR5 proteins coimmunoprecipitated from rat brain lysates (Fig. 1B). Experiments with truncated mutants of mGluR5 indicated that the membrane proximal region of mGluR5a (Ala⁸²⁶ to Gly⁹³⁴) interacted with Norbin (fig. S2A). Further studies narrowed the binding sites to two small regions, region A (Arg⁸⁵⁷ to Arg⁸⁶⁷) and region B (Gly⁸⁹³ to His⁹⁰³) (fig. S2B). Synthetic peptides covering either region A or B interfered with the interaction between mGluR5 and Norbin in cell lysates (fig. S2C). Replacement of the key amino acids in either region A (mGluR5-mut1) or B (mGluR5-mut2) or both (mGluR5-mut1/2) with alanine abolished binding of mGluR5 to Norbin (fig. S2D). Norbin-binding regions partially overlapped with identified calmodulin-binding sites (fig. S3, A and B) (8). However, mGluR5-mut1 is defective in Norbin binding (fig. S2D), but not in calmodulin binding (fig. S3C) and, therefore, was used to study the specific role of Norbin in the regulation of mGluR5 function. Homer and Norbin did not affect each other's binding to mGluR5 (fig. S3D).

Using an affinity-purified antibody to Norbin (fig. S4A), prominent expression of Norbin in adult mouse brain was observed in the hippocampus, amygdala, septum, and nucleus accumbens, with moderate expression in the dorsal striatum (Fig. 1C). This distribution of Norbin resembles that of mGluR5 (13). A synaptosomal fraction purified from mouse brain contained

both Norbin and mGluR5 (Fig. 1D). In primary hippocampal neurons, Norbin and mGluR5 were localized together in dendrites and had a punctate appearance (Fig. 1E). Double staining with antibodies to microtubule-associated protein 2 (MAP2) or spinophilin (Fig. 1F) indicated that Norbin was localized with the dendritic spine marker spinophilin.

We assessed the physiological consequence of Norbin expression on signaling through the mGluR5 receptor. mGluR5 is coupled to the heterotrimeric guanine nucleotide-binding protein (G protein) α subunit G_q that activates phospholipase C, causes generation of inositol 1,4,5-trisphosphate (InsP₃), and leads to calcium release and calcium oscillations (1, 14). After a 30-min exposure to a group I mGluR agonist (10 μ M L-glutamic acid), more inositol phosphates were formed in human embryonic kidney (HEK) 293T cells doubly transfected with mGluR5 and Norbin ($84.6 \pm 5.7\%$ increase above basal level) than in cells transfected with mGluR5 alone ($57.0 \pm 3.1\%$ increase above basal level) (fig. S4B). Activation of mGluR5 leads to extracellular signal-regulated kinase ERK1/2 phosphorylation, and this was also enhanced when Norbin was coexpressed in HEK293T cells (fig. S4C).

We tested whether Norbin transfection affected mGluR5-elicited calcium oscillations in HEK293 cells stably expressing mGluR5. The average length of the calcium oscillations was significantly longer in Norbin-expressing cells than in control cells (12.5 ± 0.8 min versus 8.2 ± 1.1 min, $P < 0.05$, Wilcoxon rank sum test) (Fig. 2, A and B). The mean number of calcium peaks was significantly higher in Norbin-expressing cells (11.2 ± 0.9 peaks; $n = 196$) than in control cells (6.7 ± 0.8 peaks; $n = 105$; $P < 0.05$, Wilcoxon rank sum test) (dashed lines in Fig. 2B). Additionally, 16% of the control cells failed to respond to (*S*)-3,5-dihydroxyphenylglycine (DHPG), whereas only 7% of the Norbin-transfected cells failed to do so [Fig. 2B (bottom), yellow and blue bars]. However, the effect of Norbin was not seen in cells expressing the Norbin-binding defective mutants, mGluR5-mut1 and mGluR5-mut1/2 (Fig. 2C).

The fact that Norbin binds to the membrane proximal region of mGluR5 prompted us to test whether Norbin might influence cell surface expression of mGluR5. The amount of cell surface mGluR5 was significantly increased in the presence of cotransfected Norbin in N2a cells (a cell line derived from mouse neural crest), whereas

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the amounts of mGluR5-mut1 or mGluR5-mut1/2 (which do not bind to Norbin) at the cell surface were not affected (Fig. 3A). When endogenous Norbin expression was decreased in primary cortical neurons with a specific short hairpin-

RNA (shRNA) (fig. S5A), the amount of mGluR5 at the cell surface was reduced (Fig. 3B and fig. S5B).

To further evaluate the role of Norbin in regulation of mGluR5 signaling, we generated

Norbin conditional knockout (KO) mice (fig. S6A) (6) with deletion of the gene for the Norbin protein specifically in the postnatal forebrain (fig. S6, B to D) (15). The abundance of mGluR5 at the cell surface was assayed in wild-type

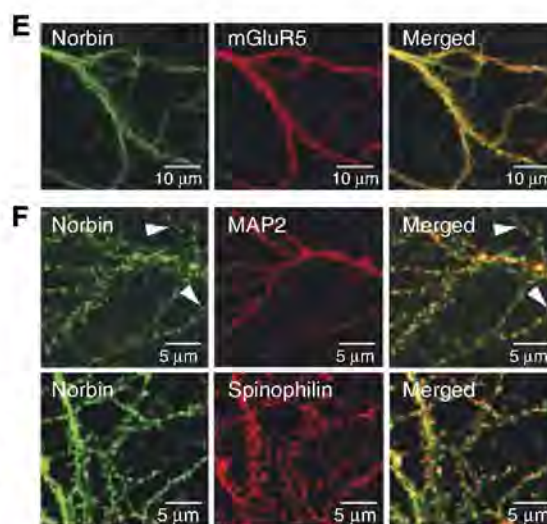
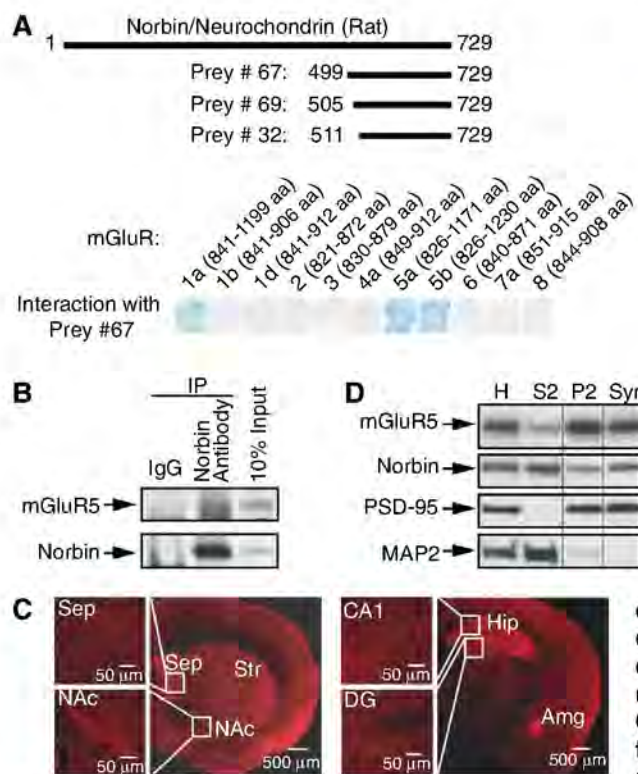
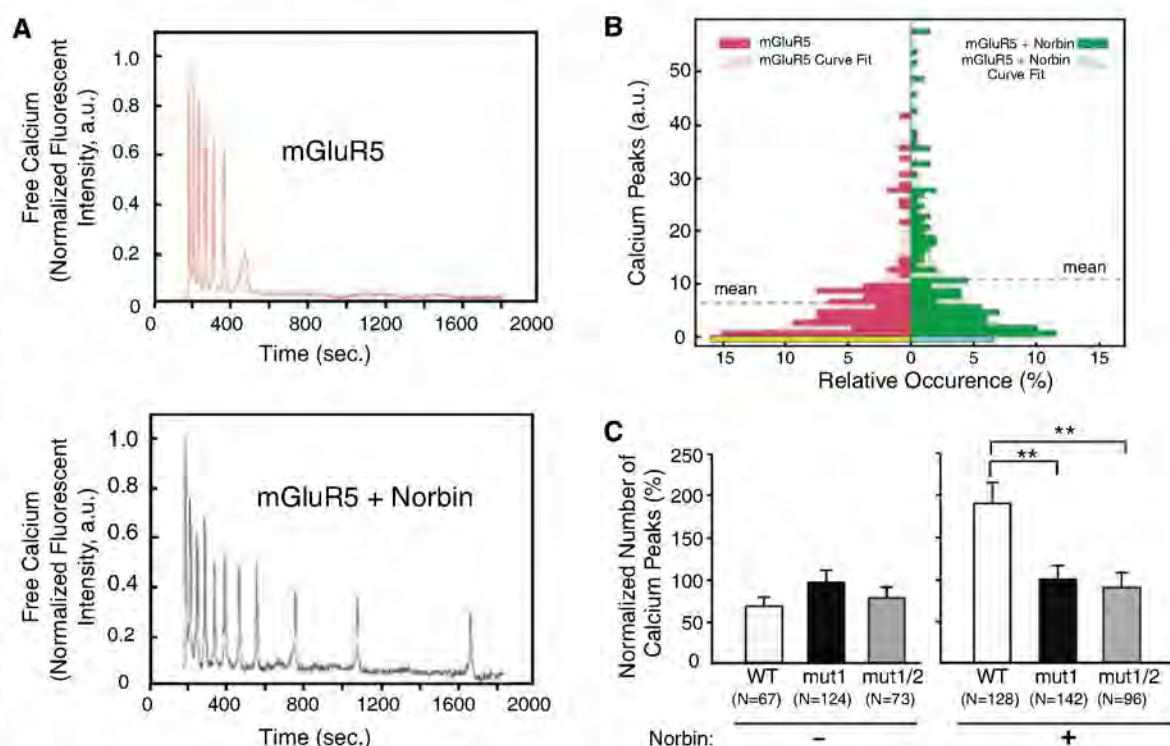


Fig. 1. Analysis of the interaction between Norbin and group I mGluRs. **(A)** (Top) Yeast two-hybrid screen using the cytoplasmic tail of mGluR5a (amino acids 826 to 1171) as bait identified three partial cDNA clones of Norbin (preys 32, 67, and 69). Numbers represent corresponding amino acid residues. (Bottom) Cytoplasmic tails of mGluR1 to 8 (as indicated) were co-expressed with the Norbin C terminus (amino acids 499 to 729) in a yeast two-hybrid system, and

their interactions were tested by β -galactosidase assay. **(B)** Rat hippocampal homogenate was subjected to coimmunoprecipitation using purified antibody to Norbin or control normal rabbit immunoglobulin IgG. Samples were analyzed by Western blotting with either mGluR5-specific (top) or Norbin-specific (bottom) antibodies. **(C)** Coronal slices (50 μ m) of adult mouse brain were immunostained with antibody to Norbin. (Insets) Septum (Sep), nucleus accumbens (NAc), and dentate gyrus (DG) are shown with higher magnifications. Other abbreviations: Str, striatum; Hip, hippocampus; Amg, amygdala. **(D)** Synaptosomal fractions (Syn) from mouse brain homogenate (H) were analyzed by Western blotting. S2 and P2, supernatant and pellet after 15,000g centrifugation. **(E and F)** Cultured 21 days in vitro (DIV 21) hippocampal neurons were fixed and double-immunostained **(E)** with antibodies to Norbin and to mGluR5 (labeled by Zenon Alexa Fluor 568) and **(F)** with antibodies to Norbin, to dendritic marker MAP2, or to the spine marker spinophilin.

Fig. 2. Effects of overexpression of Norbin on mGluR5 signaling. **(A)** HEK293 cells stably expressing mGluR5 were transiently transfected with Norbin, and shown is ratiometric calcium imaging following exposure to DHPG (10 μ M) in cells (top) not expressing or (bottom) expressing Norbin. **(B)** The relative distributions of the number of calcium peaks during 28 min of continuous exposure to 10 μ M DHPG in cells (pink) not expressing or (green) expressing Norbin. The yellow and blue bars at the bottom of the figure show the number of cells that failed to respond to DHPG. The mean numbers of calcium peaks are displayed as dashed lines. **(C)** HEK293 cells were transiently transfected with wild-type (WT) mGluR5, mGluR5-mut1, or mGluR5 mut1/2 alone (left) or together with Norbin fused with enhanced green fluorescent protein (EGFP-Norbin) (right). The number of calcium peaks during 20 min of continuous exposure to 10 μ M DHPG was recorded. Only cells responding to treatment were



included, and the data were normalized to the mean number of peaks of cells transfected with mGluR5-mut1. Data are means $\pm$ SEM (** P < 0.01, Wilcoxon rank sum test).

(*Ncdn^{Fllox/Fllox}*) and Norbin KO (*Ncdn^{Fllox/Fllox};Cre*) mice by radioligand binding. Significantly less tritiated MPEP [2-methyl-6-(phenylethynyl) pyridine hydrochloride], a specific mGluR5 antagonist, was bound to membrane fractions from Norbin KO mice than to membranes from wild-type mice (Fig. 3C), whereas total amounts of mGluR5 in the forebrain were not affected (fig.

S6E). The amount of mGluR5 on the surface of cultured primary cortical neurons from Norbin KO embryos was also significantly reduced (Fig. 3D).

mGluR5 is important for synaptic transmission and synaptic plasticity. Depletion of Norbin did not alter basal synaptic transmission or short-term plasticity in hippocampal Schaffer collateral

to CA1 synapses (fig. S7A and S7B). Activation of mGluR5 induces long-term depression (LTD) in the CA1 region (16). Norbin KO mice showed reduced DHPG-induced LTD ($85.4 \pm 1.4\%$ of pre-DHPG baseline, $n = 6$), compared with that of wild-type mice ($69.9 \pm 1.7\%$ of pre-DHPG baseline, $n = 8$) (Fig. 4A). Activation of group I mGluRs is necessary for another form of synaptic

Fig. 3. Effects of Norbin on the abundance of mGluR5 at the cell surface. **(A)** Biotinylated cell surface proteins from N2a cells were transfected with myc-mGluR5 [wild-type (WT), mut1, or mut1/2], and (top) vector or HA-Norbin and (bottom) total lysates were analyzed by Western blotting by using antibodies to Myc and hemagglutinin (HA). **(B)** shRNA-724 targeting Norbin or control shRNA was transfected into embryonic day 18 (E18) cortical neurons using a Nucleofector Kit. Five days after transfection, cell surface mGluR5 level was examined by surface biotinylation experiments. **(C)** Binding of [3 H]MPEP to membrane fractions from forebrain of wild-type (WT) and Norbin KO mice. Means $\pm$ SEM ($n = 4$, $*P < 0.05$ and $**P < 0.01$, Student's t test). **(D)** E18 primary cortical neurons isolated from wild-type and Norbin KO embryos were subjected to surface biotinylation experiments on DIV 7. Biotinylated mGluR5 was detected by Western blotting. Presenilin (PS1) was used as control surface protein, and β -actin was used as loading control. (Right) Quantification of results from four independent cultures. Means $\pm$ SEM ($*P < 0.05$, Student's t test).

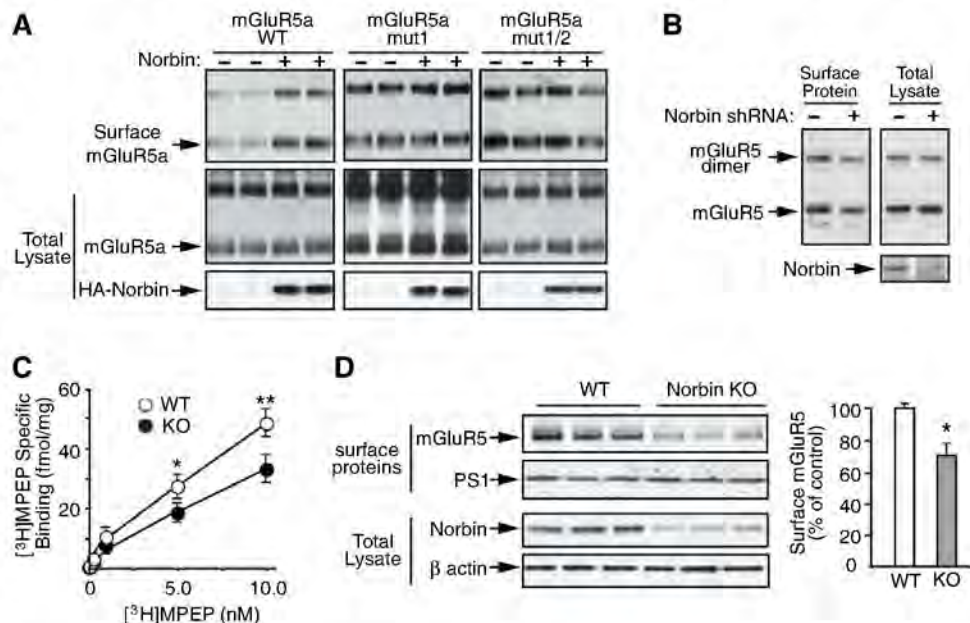
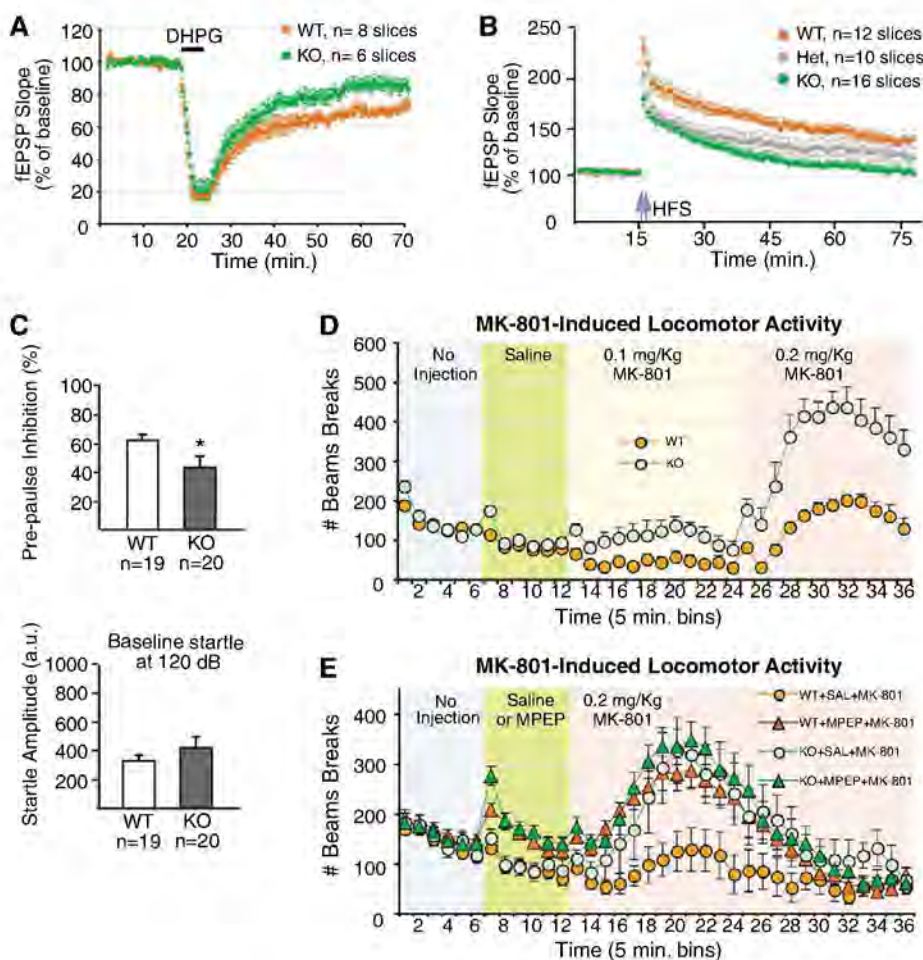


Fig. 4. Reduced DHPG-induced LTD, impaired LTP, and schizophrenia-like behaviors in Norbin knockout mice. **(A)** Averaged slopes of field excitatory postsynaptic potentials (fEPSPs) were plotted (percentage of pre-DHPG baseline) as a function of time at Schaffer collateral to CA1 synapses before and after DHPG administration ($100 \mu\text{M}$, 5 min as indicated by the black bar) treatment for Norbin WT or Norbin KO mice. Data are means $\pm$ SEM. **(B)** LTP analyzed as averaged slopes of fEPSPs at Schaffer collateral to CA1 synapses before and after tetanic stimulation (blue arrows) for WT, Norbin KO, or heterozygous (Het) mice. Means $\pm$ SEM. **(C)** (Top) PPI of startle in WT and Norbin KO. Mice received prepulses of 74 dB and pulses of 120 dB. Means $\pm$ SEM. $*P < 0.05$, Student's t test. (Bottom) Average startle magnitudes at 120 dB. **(D)** WT ($n = 8$) and Norbin KO ($n = 8$) mice were successively injected with saline and 0.1 mg/kg and 0.2 mg/kg MK-801 at the indicated times. Locomotor activity was measured by number of beam breaks. **(E)** WT and Norbin KO mice were injected with saline (WT, $n = 10$; KO, $n = 10$) or MPEP (WT, $n = 12$; KO, $n = 10$) and 0.2 mg/kg MK-801 successively at the indicated times. Locomotor activity was measured by number of beam breaks.



plasticity, long-term potentiation (LTP), in the CA1 region. Deletion of mGluR1 or mGluR5 reduces LTP (17–19). Knockout of Norbin abolished the induction of LTP in the Schaffer collateral to CA1 synapses (Fig. 4B).

Prepulse inhibition of startle (PPI) is a phenomenon in which a reaction (startle) induced by a strong startling stimulus (pulse) is inhibited by a weaker prestimulus (prepulse). Defects in PPI reflect abnormalities of sensorimotor gating, a clinically important feature of schizophrenia (20). mGluR5 positively regulates the function of the NMDA (*N*-methyl-D-aspartate) receptor in the CNS (21). Consistent with the NMDA hypofunction hypothesis of schizophrenia (22), mGluR5 KO mice display a disruption in PPI (23). We therefore tested PPI in Norbin KO mice. Compared with wild-type littermate controls, Norbin KO mice showed impaired PPI at a prepulse intensity of 74 dB (4 dB above background noise) (Fig. 4C, top). There was no significant difference in baseline startle response between these two groups (Fig. 4C, bottom).

Similarly to mice treated with the mGluR5 antagonist MPEP (24–26), Norbin KO mice showed more locomotor activity in response to MK-801, an NMDA antagonist, than did wild-type controls. There was no difference in baseline locomotor activity observed between wild-type and Norbin KO mice (Fig. 4D and fig. S8A). MPEP augmented the locomotor-stimulating effect of MK-801 by 109% in WT mice, but had

no effect in Norbin KO mice (Fig. 4E and fig. S8B). These results strongly suggest that the hypersensitivity of Norbin KO mice to MK-801 is due to reduced mGluR5 signaling.

In summary, a variety of cell biological, electrophysiological and behavioral studies indicate that Norbin is an important endogenous modulator of mGluR5 and may provide a therapeutic target for schizophrenia.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5959/1554/DC1

Materials and Methods

Figs. S1 to S8

References

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Human Resources

Anatomy Chair Search Committee
Kirkville College of Osteopathic Medicine
A.T. Still University of Health Sciences
800 W. Jefferson Street
Kirkville, MO 63501

Electronic applications can be sent to e-mail: hr@atsu.edu. The position is available July 1, 2010. Review of applications will begin immediately and continue until the appropriate individual is found.

A.T. Still University of Health Sciences does not discriminate on the basis of race, color, religion, national origin, sex, gender, sexual preference, age, or disability in admission or access to, or treatment or employment in its programs and activities.

INSTRUCTOR POSITION available within the Division of Hematology/Oncology and Palliative Care within the Department of Internal Medicine of the Virginia Commonwealth Health Sciences System to conduct research involving the investigation of molecularly targeted agents in hematologic malignancies. The laboratory has a strong track record of translational initiatives based upon rational combinations of targeted agents, including proteasome, histone deacetylase, and tyrosine kinase inhibitors, in leukemia, multiple myeloma, and non-Hodgkin's lymphoma. The laboratory is closely aligned with the Massey Cancer Center, an NCI-designated cancer center, and its Cancer Cell Signaling program.

The candidate must have an M.D. degree with post-doctoral research experience utilizing advanced molecular techniques, and experience working in the area of translational studies aimed at developing a basis for using novel signal transduction and other targeted agents including proteasome inhibitors in the treatment of hematologic malignancies, particularly in the areas of multiple myeloma and leukemia. The candidate must also have a successful publication record and the ability to train others to perform advanced molecular biology techniques. The candidate must have seven to eight years of experience, have the ability to design, conduct, and supervise a research project, and must be a first author on multiple publications in peer-reviewed scientific journals. Experience in stable and transient gene transfection, kinase assays, immunoprecipitation assays, multiple myeloma and leukemia cell biology, apoptosis regulation, and genetic engineering is highly desirable.

Submit letter of interest, curriculum vitae, brief description of career plans, and three references to: Steven Grant, M.D., c/o Karen Scott, Division of Hematology, Oncology and Palliative Care, P.O. Box 980230, Richmond, VA 23298-0230. *Virginia Commonwealth University is an Equal Opportunity Affirmative Action Employer. Women, minorities, and persons with disabilities are encouraged to apply. Position open until filled.*

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Candidates should send curriculum vitae, description of research interests and accomplishments to:

Heidi E. Hamm, Chair

Department of Pharmacology
Vanderbilt University School of Medicine
442 Robinson Research Building
Nashville, TN 37232-6600
Telephone: 615-343-3533; fax: 615-343-1084
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Investigators in the Center for Cardiovascular Sciences have opportunities for collaboration with engineers and bioengineers at Rensselaer University and SUNY Albany College of Nanosciences. The area offers diverse cultural and recreational attractions, outstanding primary and secondary schools, and an attractive cost of living. For further information about Albany Medical Center, the College, and Center for Cardiovascular Sciences, please visit website: <http://www.amc.edu>.

Applicants should submit curriculum vitae, description of research interests and plans, and the names of at least three references by March 1, 2010, to:

Dr. Harold A. Singer

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Corrigendum to advertisement for recruitment to the post of Director, ARI, Pune, published in *Science (USA)* Volume 322, 31 October 2008

Maharashtra Association for the Cultivation of Science (MACS) had invited applications for the post of Director at the Agharkar Research Institute, Pune, India (see details at website: <http://www.aripune.org>). The prerequisites for this position have changed; for details please refer to our website. Interested candidates may submit their applications by 31 December 2009 addressed to: Chairman, Institute Council, Agharkar Research Institute, G.G. Agarkar Road, Pune - 411 004, India. Applications received earlier would be considered and hence those who have applied earlier need not reapply.

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Argonne Distinguished Fellow for Life Sciences at the Advanced Photon Source

The Advanced Photon Source at Argonne National Laboratory is the brightest source of x-rays in the western hemisphere and attracts nearly 3,500 scientific users each year, almost half of which are life scientists. We are seeking an eminent scientist to fill the new position of Argonne Distinguished Fellow for Life Sciences at the APS.

The Distinguished Fellow for Life Sciences will serve as an advisor to the Director of the Advanced Photon Source on issues related to life sciences research at the APS and as an advocate for the life sciences community. He/She will be expected to coordinate outreach at the APS for all life science-based work, assist in the management of the General User program for life sciences, work with the life science-based Collaborative Access Teams (CATS) to improve the quality and productivity of the life science research programs at the APS, and to integrate their programs into the APS. The Distinguished Fellow may carry out his/her research at the APS or elsewhere. The percentage of time for personal research will be negotiated on a case-by-case basis and a joint appointment with another Argonne Division or with a local university is encouraged.

The Distinguished Fellow (710 career level) is Argonne National Laboratory's highest scientific and engineering rank. Award of this rank confers the title Argonne Distinguished Fellow, which is comparable in stature to an endowed chair at a top-ranked university and recognizes exceptional contributions in a person's field. Argonne Distinguished Fellow for Life Sciences at the APS should be internationally recognized in the application of synchrotron radiation to the life sciences, with a relevant PhD and 15-20 years experience. The individual should have demonstrated leadership and management abilities, at least at the level of a group or department, and the ability to articulate a vision for life sciences at APS. The selected candidate for hire at this level will be subject to approval by the Laboratory Directors Oversight Committee for Hires and Promotions (LDOC-HP) peer review requiring preparation and submission of a comprehensive case package.

Argonne National Laboratory is a multi-program laboratory operated by the UChicago Argonne, LLC for the U.S. Department of Energy. Argonne's site is located about 25 miles southwest of Chicago on a beautiful 1500-acre campus-like environment. Interested candidates should send a detailed CV along with a list of publications, and the names and addresses of three references through the Argonne website at <http://www.anl.gov/jobs> job search for requisition 315410 XSD. Review of applicants will begin January 2010 and will continue until the position is filled.

For additional technical information, please contact Dr. Dennis Mills, APS Deputy Director for X-ray Science at dmm@aps.anl.gov.

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The Biology Department invites applications for one or more tenure-track or tenured positions at the level of Assistant, Associate or Senior Scientist. These are regular full-time positions and are eligible for benefits.

We seek exceptional candidates from the biological, mathematical and physical sciences to complement our existing interdisciplinary strengths in oceanography and marine ecology. Both theoretical and empirical approaches are welcome.

Candidates in all areas are welcome to apply; research topics of particular interest include: **Climate Change:** Scientists who conduct research on the effects of climate change (including ocean acidification) on populations, communities and ecosystems, especially in the context of large-scale or global climate processes and models.

Population Genetics: Scientists who use population genetics to address questions about the structure, dynamics, conservation or biogeography of marine populations.

We expect to hire at the Assistant Scientist level, but we will consider an appointment at a higher level for an exceptionally qualified candidate. Successful candidates will be expected to develop an internationally recognized and externally funded research program. They also have the opportunity to advise graduate students and teach courses at MIT/WHOI Joint Program in Oceanography. While members of the Institution's Scientific Staff are expected to provide for their salaries from grants and contracts, the Institution provides salary support when no other funding is available. Candidates hired at the Assistant Scientist and Associate Scientist without tenure levels receive an initial appointment for four years with salary guaranteed.

Candidates should include a 2-3 page research statement, a CV with the names and addresses of four references, and copies of up to three relevant publications. The application review process will begin on **January 15, 2010**.

HOW TO APPLY: If you are an interested applicant, there is a two-step process to apply for this position. *You will not finish the application process until BOTH Steps 1 and 2 are completed:*

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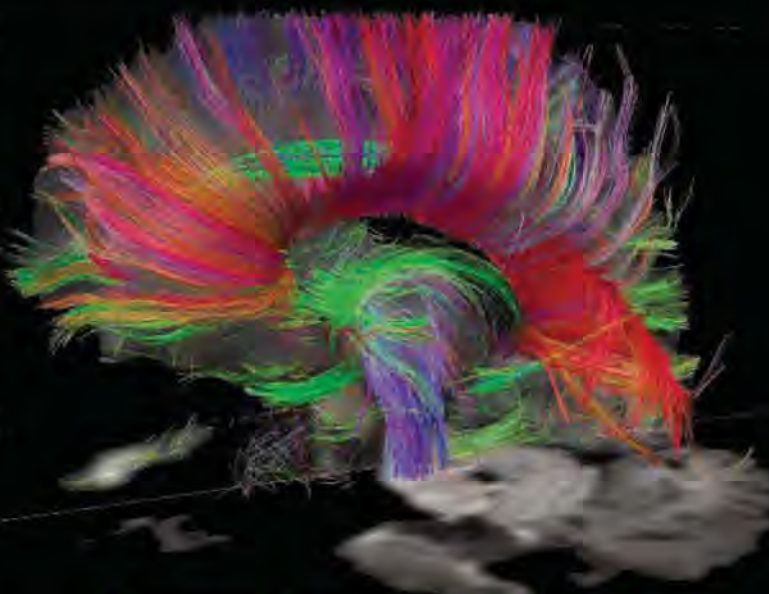
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Successful candidates will be accomplished neuroscientists who have a strong commitment to the advancement of basic, translational and clinical research, a demonstrated record of research program management, and outstanding leadership skills. As Program Directors, they will conceive, develop, and oversee cutting-edge research programs in their areas of expertise.

Experience in basic neuroscience (genetic, molecular, cellular, circuit or systems level), translational neuroscience (therapy or diagnostic development) and/or clinical research or clinical practice in academic or for-profit institutions, is highly desirable. Applicants must have the capacity to interact effectively with national and international organizations and with individuals who represent wide-ranging views and competing priorities.

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National Institute of Neurological Disorders and Stroke

Applicants must possess a Ph.D. and/or M.D. degree, have experience in research program oversight, and demonstrate extensive involvement in independent and collaborative projects involving neurodegeneration, stroke, neural biomedical engineering or related fields.

Application Information

Applications may be submitted beginning 11/30/2009 to 01/31/2010. Application instructions can be found at the USAJobs Web Site (<http://www.usajobs.gov/>), by searching on Vacancy Announcement for Health Scientist Administrator GS-0601-13/14: NINDS-09-366942-DE or Medical Officer, GS-0602-13/14/15: NINDS-09-366806-DH. Applicants must be U.S. citizens. The salary ranges from \$86,927 to \$153,200 per annum and is commensurate with experience and qualifications. For more information, please contact Dr. Robert Finkelstein at finkelsr@ninds.nih.gov.





香港中文大學
THE CHINESE UNIVERSITY OF HONG KONG

Applications are invited for:-

Director and Professor, School of Life Sciences (Ref. 08/227(665)/2)

The Chinese University of Hong Kong, founded in 1963, aspires to be acknowledged regionally and internationally as a first-class comprehensive research university. The University offers a broad spectrum of programmes up to PhD level in various disciplines organized under eight Faculties (viz. Arts, Business Administration, Education, Engineering, Law, Medicine, Science and Social Science), with a team of over 2,000 full-time teaching and research staff. In 2009-10, undergraduate and postgraduate enrolment in publicly-funded programmes stands close to 11,500 and 3,100 respectively (<http://www.cuhk.edu.hk>).

The Faculty of Science comprises six Departments: Biochemistry, Biology, Chemistry, Mathematics, Physics, and Statistics, and one School: Chinese Medicine, and also offers a number of multidisciplinary programmes: Cell and Molecular Biology, Environmental Science, Food and Nutritional Sciences, Molecular Biotechnology, Mathematics and Information Engineering (double degree), Quantitative Finance and Risk Management Science (double major), and Risk Management Science. The Faculty has 300 full-time teaching and research staff, 1,600 undergraduate students and 500 postgraduate research students. Detailed information on the Faculty is available at <http://www.cuhk.edu.hk/sci/>.

A new School of Life Sciences will be established within the Faculty of Science through re-organization of teaching and research programmes in the existing Departments of Biochemistry and Biology. The School will ultimately have more than 40 academic staff, about 900 undergraduate and 300 postgraduate students. World-class research facilities are available. The School will create synergies in research and scholarship, and provide the institutional framework for taking advantage of exciting new opportunities in modern life sciences, both within the Faculty of Science and also in collaboration with other Faculties.

The School will offer six undergraduate major programmes and corresponding research postgraduate programmes in Biochemistry, Biology, Cell and Molecular Biology, Environmental Science, Food and Nutritional Sciences, and Molecular Biotechnology; and also two taught MSc programmes: Biochemical and Biomedical Sciences, and Nutrition, Food Science and Technology. The School will enter a period of expansion with the introduction of the Cell and Molecular Biology programme in 2009-10 and the revival of a four-year normative undergraduate curriculum in 2012-13.

Applicants should be academics with (i) an outstanding record of scholarship appropriate for a senior appointment at professor level in a discipline of life sciences; (ii) an appreciation of the breadth and depth of research and educational activities in life sciences, in particular the opportunities ahead for cutting-edge research; (iii) experience in leading well-funded, internationally-recognized, and preferably multi-disciplinary collaborative research programmes; (iv) demonstrated leadership and management ability at an appropriate level in higher education institutions; (v) long-term vision for the development of the School into a centre of research excellence; and (vi) excellent interpersonal and communication skills.

As the academic and executive head of the School reporting to the Dean of the Faculty of Science, the appointee will (a) be responsible for the vision and stewardship of the School; (b) undertake overall responsibility for academic, financial, staff and student matters; and (c) provide strong leadership in the re-organization process, particularly in planning and leading research directions of the School. Appointment will initially be made on contract basis for up to five years commencing as soon as possible, which, subject to mutual agreement, may lead to longer-term appointment or substantiation later. The selected candidate will also be offered a full professorship appointment in a subject related to his/her own speciality area of scholarly interests, to be held concomitantly with the Directorship of the School.

Consideration of applications/nominations will begin in January/February 2010 and will continue until the post is filled. The University reserves the right to fill the post by invitation.

[Note: Those who have responded to the previous advertisement for this post (under the same Ref. no.) need not re-apply on this occasion.]

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Application Procedure

Please send full resume, copies of academic credentials, a publication list and/or abstracts of selected published papers, together with names, addresses and fax numbers/e-mail addresses of three referees to whom the applicants' consent has been given for their providing references (unless otherwise specified), to the Personnel Office, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong (fax: (852) 2696 1462). The Personal Information Collection Statement will be provided upon request. Please quote the reference number and mark 'Application - Confidential' on cover.



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The Max Planck Institute of Immunobiology, an internationally renowned research institute in the area of Immunobiology and Epigenetics in Freiburg, Germany, seeks a

Manager for the recently established Fly Facility

The position is available immediately and will be initially for two years with the possibility of an extension.

We are looking for a motivated individual with strong people and communication skills, who would like to take on the responsibility to run our new fly facility. Candidates should hold a PhD and have a strong background in molecular biology approaches and various fly techniques. In particular, previous experience with fly genetics, fly injections and day-to-day handling of flies is absolutely essential. Additional experience in preparing polytene chromosome spreads, imaging, handling large fly populations for biochemical analysis or screening purposes will be advantageous. The successful candidates will also be expected to teach and train new users of the fly facility. Next to running the facility, there will be an option to participate in scientific projects in the Akhtar laboratory in the field of chromatin and epigenetics (http://www.immunbio.mpg.de/home/research/biol/laboratory_akhtar/index.html).

Salaries will be based on previous experience according to TVöD guidelines. A childcare facility is directly attached to the MPI of Immunobiology. Handicapped applicants with equal qualifications will be given preferential treatment. The Max Planck Society seeks to increase the number of women in areas, where they are underrepresented, and therefore explicitly encourages women to apply.

Candidates should send their application indicating the reference number 011109 via e-mail to weigold@immunbio.mpg.de or by regular mail to the Personnel Department, Max Planck Institute of Immunobiology, Stübeweg 51, D-79108 Freiburg, Germany.

More information about our institute can be found on <http://www.immunbio.mpg.de>.

Applications will be accepted until January 15th, 2010.

Faculty Positions in Cancer Biology/Therapeutics and Vascular Biology/Drug Research

The newly created Department of Biomedical Sciences, Texas Tech University Health Sciences Center (TTUHSC), Amarillo, TX seeks applicants for two new tenure-track faculty positions at the Assistant/Associate Professor level. The successful candidates will join a vibrant and expanding group of extramurally funded biomedical researchers (<http://www.ttuhsc.edu/sop/biomedicalsciences>) who are part of the University Cancer Biology and Vascular Biology Centers.

Applicants for the Cancer Biology position must have a doctoral degree with research experience in any aspect of cancer biology, chemotherapeutics or drug discovery. Applicants for the Vascular Biology position must have a doctoral degree with research experience in any aspect of vascular biology. The applicants at the Assistant Professor level are expected to develop an extramurally funded research program, whereas applicants at the Associate Professor level should have current peer-reviewed funding and track record of independent research and publishing. The successful candidates will be expected to teach Pharm.D. and Ph.D. students. Competitive salary, start-up package, and laboratory space are available. Applicants should submit documents online at <http://jobs.texastech.edu> (job requisition numbers: 80378 (Cancer Biology), 80379 (Vascular Biology)). Please include curriculum vitae, a summary of research accomplishments/interests, future directions, teaching philosophy and names and addresses of three references. TTUHSC in Amarillo includes the School of Pharmacy (Department of Biomedical Sciences, Department of Pharmaceutical Sciences and Pharmacy Practice), School of Medicine, The Laura Bush Woman's Health Research Institute and the Harrington Cancer Research Center. The Biomedical Sciences department currently has 11 full-time faculties with interest in cancer biology, vascular biology, and pharmaceutical research. For Cancer Biology position contact the search committee chair, Dr. Sanjay K. Srivastava. E-mail: sanjay.srivastava@ttuhsc.edu. Telephone: (806) 356-4750 ext 224 and for Vascular Biology position contact Dr. Jay Gunaje. E-mail: jayarama.gunaje@ttuhsc.edu Phone: 806-356-4015, Ext. 249.

TTUHSC is an Equal Opportunity/Affirmative Action Institution.
Minorities and women are encouraged to apply.

TO DO THE WORLD A FAVOUR WE NEED SEVEN OF ITS BEST RESEARCHERS IN THE BASIC SCIENCES



If taking initiatives is easy, taking responsibility for them is somewhat harder. But having initiated eight areas of advance to promote a better world, we are now ready to invest in the fundamental sciences – the cornerstones of our research. We seek seven ambitious women or men for Assistant Professorships and PhD student positions in fields that will contribute to the basic sciences of tomorrow, and fortify our areas of advance and vision of sustainability.

Each assistant professor will have the chance to design a unique and autonomous programme networking with specialists and students across various borders. Additionally, successful candidates will receive a generous support package.

TWO ASSISTANT PROFESSORS TO THE BASIC SCIENCES

Are you working in pure physics, mathematics, astronomy, chemistry or computer science? Would your area of research benefit from an environment where scientists are encouraged to cross the borders that otherwise impede progress? Will your insights help to develop the foundations of a university that seeks excellence in both pure and applied sciences? In this case, we look forward to hearing from you.

FIVE PHD STUDENTS TO THE BASIC SCIENCES

Are you looking for the ultimate PhD position to nurture a promising research career in a basic science? Successful candidates will have an exceptional opportunity to design a programme taking full advantage of our many strengths.

Chalmers is a highly progressive university situated in Göteborg, Sweden. From this beautiful and dynamic part of the world we have become known locally and globally for education, research and innovation with a wide range of applications. Our many strengths are reinforced by a foundation of world-renowned research in the basic sciences. As we strive to use science and technology to advance sustainability in the world around us, we are reminded of the motto of our founder, William Chalmers. Avancez!



To know how you might qualify for one of these exceptional positions, please visit us at

www.chalmers.se/basicsciences

CHALMERS
UNIVERSITY OF TECHNOLOGY



Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

PAUL SCHERRER INSTITUT



Professor of Electrochemistry and Head of the Electrochemistry Laboratory

The Department of Chemistry and Applied Biosciences at ETH Zurich (www.chab.ethz.ch) and the Paul Scherrer Institute (www.psi.ch) invite applications from candidates who have demonstrated an exceptional potential to develop an innovative and collaborative research program at the interface between electrochemistry and electrochemical engineering.

The new professor will hold the position of Head of the Electrochemistry Laboratory at the Paul Scherrer Institute (PSI) in Villigen, in a joint appointment between ETH Zurich and the PSI. The professorship will be associated with the Laboratory of Physical Chemistry at ETH, and will contribute to the teaching within the Department. Experimental activities in the fields of electrochemical energy conversion and storage, including fuel cells and advanced batteries, will be carried out at the Paul Scherrer Institute and will take advantage of the specific opportunities offered by the large facilities of the PSI. Projects are carried out in national and international collaborations, spanning the full range from fundamental investigations of processes at electrochemical interfaces to system applications of industrial relevance.

Requirements include an internationally recognized research program and excellence in teaching; industrial experience is an advantage. The new professor will be expected to teach undergraduate level courses (German or English) and graduate level courses (English).

Please submit your application together with a curriculum vitae, a list of publications as well as a list of current projects and a research plan to the President of ETH Zurich, Prof. Dr. R. Eichler, ETH Zurich, Raemistrasse 101, 8092 Zurich, Switzerland, no later than February 28, 2010. With a view toward increasing the number of female professors, ETH Zurich specifically encourages qualified female candidates to apply.



www.ox.ac.uk/jobs

Programme Director of the Futures Programme



Salary in the region of £100k

Smith School of Enterprise and the Environment

Applications are invited for the post of Programme Director of the Futures Programme in the Smith School of Enterprise and the Environment in the Social Sciences Division of the University of Oxford. Founded in October 2008, the School provides an inter-disciplinary centre for research, business and government leaders to seek solutions to the most pressing issues of the 21st century. The Founding Director is Professor Sir David King, formally Chief Scientific Advisor of the UK Government.

The primary task of the post-holder will be to establish a new Futures Programme in the School, working closely with private sector companies, government policy makers, the wider academic community and other futures researchers at Oxford to conduct a series of futures work projects associated with the 21st century environmental challenges.

The post holder will be an internationally leading futures/foresight expert, who will have the vision and organisational capacity to establish a world-leading futures group which will have a major impact on private sector companies and governments in establishing low-carbon, sustainable growth capabilities.

Further particulars and full application details are available from the Administrator at personnel@smithschool.ox.ac.uk or on the School's website at <http://www.smithschool.ox.ac.uk/vacancies>

Please quote reference LD-09-013 in all correspondence.

The closing date for applications is Monday 4 January 2010.

Committed to equality and valuing diversity

Joint Department of Biomedical Engineering The University of North Carolina at Chapel Hill and North Carolina State University at Raleigh



152 MacNider Hall, Chapel Hill, NC 27599-7575
(919) 966-1175; (919) 966-2963 fax
<http://www.bme.unc.edu>



2147 Burlington Labs, Raleigh, NC 27695-7115
(919) 515-5252; (919) 513-3814 fax
<http://www.bme.ncsu.edu>

Faculty Position at the University of North Carolina - North Carolina State University Joint Department of Biomedical Engineering

The Joint Department of Biomedical Engineering at the University of North Carolina and North Carolina State University is seeking highly qualified candidates for a full-time, permanent, tenure-track faculty position at the level of assistant/associate professor. The desired candidate will have a demonstrated research expertise that will complement the Department's current excellence in microfluidics, and bioanalytical microdevices. The Joint Department consists of 39 primary core faculty members evenly balanced between the two campuses, and approximately 60 affiliated program faculty. For more information see our website: <http://www.bme.unc.edu>.

Applicants should possess a Ph.D. or M.D. degree, or equivalent, with at least two years of postdoctoral research experience, a scholarly publication record, and fluency in both spoken and written English. Apply online at jobs.unc.edu/1002136 and attach a curriculum vitae, a statement of research and teaching objectives, and names of at least four professional references. For full consideration, please submit application materials by **January 1, 2010**. Application review will continue until a suitable candidate is found.

UNC Chapel Hill and NC State University are Equal Access/Equal Opportunity Employers and institutions. Minorities and females are strongly encouraged to apply. For persons needing accommodations during the application process, please contact (919) 515-3148.



The
**Max Planck Institute for
Terrestrial Microbiology**

will host a Symposium in search of a

Director

of a new department

“Synthetic Cells”

to be held in Marburg on the 1st of March 2010.

The Institute is committed to basic research on microorganisms. At present, it has three research departments - Microbial Ecology & Biogeochemistry (Director: Dr. R. Conrad), Organismic Interactions (Director: Dr. R. Kahmann), and Ecophysiology (Director: Dr. L. Sogaard-Andersen). The new department will be a full department of the Institute and will be integrated into the LOEWE Center for Synthetic Microbiology (SYNMIKRO) which is a collaborative project between the Philipps-Universität Marburg and the Max Planck Institute for Terrestrial Microbiology. SYNMIKRO is committed to basic research in synthetic biology and focuses on all organizational levels of microbial function, from synthetic regulatory circuits, genetic codes, and metabolic pathways to synthetic cells and synthetic microbial communities. SYNMIKRO will comprise three full departments and four independent junior groups along with several university groups. It will be accommodated in a new building on Campus Lahnberge and will include a state-of-the-art infrastructure.

We invite applications for the Symposium from scientists with an outstanding track record in basic microbiological research and with documented experience in the new research field of synthetic biology using and/or developing rational design principles based on e.g. system biology approaches. Topics of interest include but are not limited to protocells, cells with synthetic genomes and cells with minimal genomes. The successful candidate has a commitment to actively participate in establishing SYNMIKRO, and is expected to collaborate closely with existing experimental and theoretical groups and to be active in existing and planned collaborative research initiatives (SFB, FOR and GRK). The Max Planck Society is an equal opportunity employer, and particularly encourages women to apply.

Scientists who wish to participate in the Symposium are invited to send their application to synmikro@mpi-marburg.mpg.de. The application should contain the following information in a single pdf document:

- (1) Cover letter
- (2) Curriculum vitae, including a list of publications
- (3) Summary of previous research achievements
- (4) Research plan
- (5) Statement of teaching interests and experience
- (6) Previous and current grant funding

Applications should be received by 18th of January 2010. Informal inquiries can be directed to

Prof. Dr. Lotte Sogaard-Andersen, Managing Director, Max Planck Institute for Terrestrial Microbiology, sogaard@mpi-marburg.mpg.de or
Prof. Dr. Bruno Eckhardt, Managing Director, SYNMIKRO, Philipps-Universität Marburg, director@synmikro.uni-marburg.de.

The Philipps-Universität Marburg and the Max Planck Institute for Terrestrial Microbiology will together establish a new centre for Synthetic Microbiology, SYNMIKRO. Supported by the state of Hessen within its excellence program LOEWE, SYNMIKRO will focus on basic research in synthetic biology on all levels of microbial function, ranging from the development of regulatory circuits, genetic codes and metabolic pathways to the synthesis of new minimal cells and microbial communities. In addition to the 26 groups already present, a department at the Max Planck Institute, two chairs at the university, four junior groups and several state-of-the-art infrastructural units will be established on the Campus Lahnberge in Marburg.

The Philipps-Universität Marburg is searching for outstanding scientists to fill the following positions:

The Faculty of Chemistry invites applications for the following position:

**Professorship (W3) in
Molecular and Cellular
Biochemistry of Microorganisms**

Candidates should have an outstanding record in the analysis of central biological processes using also quantitative methods. Potential research areas include complex regulatory networks in Microorganisms or the analysis of spatio-temporally resolved processes. The position holder is required to teach biochemistry courses in the Faculty of Chemistry.

The Faculty of Biology invites applications for the following position:

**Professorship (W3) in Comparative
Genomics of Microorganisms**

The successful candidate is expected to combine theoretical comparative genome analysis with experimental biological methodologies in prokaryotic or eukaryotic Microorganisms. Possible fields of research include identification of functions and experimental characterization of unknown gene products and optimizing the genetic layout of microbial minimal cells. Full participation in teaching introductory and advanced degree courses in the Faculty of Biology, especially in the field of molecular microbiology, is required.

Candidates for both positions should have an outstanding record in teaching and research as well as experience in the procurement and implementation of externally funded projects.

The conditions of employment are set down in §§ 70, 71 of the Hessen State Law on Higher Education (HHG). Philipps-Universität Marburg places great importance on the intensive mentoring of students and doctoral candidates; therefore, the regular presence of the teaching staff is required.

Philipps-Universität is an equal opportunity employer, and applications from women are encouraged. Applications from candidates with children are welcome - the Philipps-Universität is dedicated to being a family-friendly university. Qualified disabled people are also encouraged to apply.

Applications should be submitted by January 18th, 2010 to ausschreibungen@verwaltung.uni-marburg.de. The application should contain the following information in a single pdf document: (1) cover letter, (2) curriculum vitae, including a list of publications, (3) summary of previous research achievements, (4) research plan, (5) statement of teaching interests and experience and (6) previous and current funding.

Informal inquiries can be directed to Prof. Dr. Bruno Eckhardt, Managing Director SYNMIKRO, Philipps-Universität Marburg, director@synmikro.uni-marburg.de.

NORTHEASTERN UNIVERSITY: INVESTING IN EXCELLENCE

Northeastern University's response to these challenging times is to continue to invest in our core values: excellence in teaching and research. Be a part of it.

This year, Northeastern is hiring more than fifty tenure-line faculty members as we orient our research agenda to three grand global challenges—health, security, and sustainability. Within those overarching themes, we seek distinguished candidates for interdisciplinary faculty appointments in areas including:

Biomedical technology and neuroscience

Experimental or computational movement neuroscience

Nano/biological engineering

Biomedical or biological electrical engineering

Drug discovery and delivery

Bioinformatics

Regenerative medicine

Networks and Complex Systems

Network science and epidemic processes

Mathematics and computer science

Security

Information assurance and cyber security

Sustainability

Energy systems

Environmental engineering

For information on these and other outstanding opportunities, visit www.northeastern.edu/facultypositions.

Northeastern University is an Equal Opportunity, Affirmative Action Educational Institution and Employer, Title IX University. Northeastern University particularly welcomes applications from minorities, women and persons with disabilities. Northeastern is an E-Verify Employer.

Forty-three new tenured and tenure-track faculty members joined Northeastern this academic year. To meet them, visit www.northeastern.edu/facultyhires.



Northeastern

Boston, Massachusetts



IN 2010
CNRS IS RECRUITING

TENURED RESEARCHERS IN ALL FIELDS OF SCIENCE

- MATHEMATICS • PHYSICS
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- CHEMISTRY
- SCIENCE AND TECHNOLOGY OF INFORMATION AND ENGINEERING
- UNIVERSE AND EARTH SCIENCE
- ENVIRONMENT AND SUSTAINABLE DEVELOPMENT
- LIFE SCIENCES • HUMANITIES AND SOCIAL SCIENCES

CNRS encourages junior and senior scientists from around the world to apply for its tenured researcher positions.

CNRS provides an enriching scientific environment:

- numerous large-scale facilities
- highly skilled technical support
- multiple international and interdisciplinary networks
- access to university research and teaching
- lab-to-lab and international mobility

Application forms and further information will be available online at www.cnrs.fr in December 2009



OLD DOMINION UNIVERSITY

MICROBIOLOGY, IMMUNOLOGY
OR MOLECULAR CELL BIOLOGY
FACULTY POSITIONS

The Department of Biological Sciences at Old Dominion University is recruiting for mid to senior level faculty in the area of Microbiology, Immunology or Molecular Cell Biology. To be considered, investigators must have a strong record of research accomplishments as determined by: publications in top quality journals, peer reviewed grant support, and evidence of high quality teaching. Preference will be given to candidates whose research complements existing programs in the Department and/or College. The successful candidates are expected to have national/international recognition, strong record of publications and a research program consistently funded by peer-reviewed research grants. State support and a competitive start-up package will be provided. The successful candidates will be expected to contribute to our teaching of undergraduate students, as well as to develop graduate courses in their area of expertise.

Applications should include a curriculum vitae, a summary of research and teaching experience, statement of current research interests and future plans, as well as the names, addresses, e-mail address, and phone numbers of 4 references. All applicants must have a Ph.D. and/or M.D. or equivalent. Please send applications and nominations to the Search Committee (MICB) at MICB@odu.edu. Review of applicants will begin immediately and continue until the positions are filled. The anticipated start date for these positions is July 2010. The Department receives substantial support from state funds and from research grants from federal and other agencies. The Department has strong graduate programs which currently have over 100 students, including a Ph.D. program in biomedical sciences and a Ph.D. program in ecology.

Old Dominion University is an Affirmative Action/Equal Opportunity institution and requires compliance with the Immigration Reform and Control Act of 1986.



R&D OPPORTUNITIES AT PIRAMAL LIFE SCIENCES, MUMBAI, INDIA

Piramal Life Sciences Limited (PLSL) is an independent research-driven drug discovery company. PLSL has state-of-the-art R&D laboratories in Mumbai, India and over 300 scientists engaged in drug discovery and development, focusing on four therapeutic areas – cancer; diabetes; inflammation and infectious diseases. The company provides an open and employee friendly culture and encourages rigorous and high quality research work. To strengthen our R&D team, we are looking for committed, dynamic and ambitious R&D professionals with a proven track record and a flair for innovative research, to take up challenging responsibilities in the following areas of expertise/specialization:

Cancer Biology Scientists – Job code PLSL-001

Candidates should have a Ph.D in Cell Biology/Biochemistry/Cancer Biology and 3-6 years of cancer drug discovery experience. The job involves leading and managing drug discovery project/s for cancer; identification, evaluation and implementation of new cancer targets. Major responsibilities will include establishment of target-driven primary and secondary screens, and conducting mechanistic studies to understand the mode-of-action of drug candidates. Hands on experience in vivo models of cancer will be an added advantage.

Molecular Biology/Assay Development Scientist – Job code PLSL-002

The candidate should have a Ph.D in Molecular Biology/Cell Biology/Biochemistry/Biotechnology and 5-8 years of industry experience. The job involves gene cloning, expression and purification of proteins as required for various screening groups, developing biochemical and cell based assays for drug discovery programs. The candidate must be proficient in routine and advance molecular biology techniques, biochemistry and assay development, be able to trouble shoot processes, develop inter and intra project collaborations, mentor, manage and lead a group of 6-8 researchers.

Drosophila – Zebrafish Geneticist/ Molecular Biologist – Job code PLSL-003

The candidate should have a Ph.D in biological sciences with specific experience and expertise in either drosophila or zebrafish genetics. The job will entail establishing primary and secondary assays utilizing these model organisms for drug and target discovery.

Preformulation Scientists – Job code PLSL-004

Positions are open at various levels. The candidate should have a Ph.D with 2 or more years of experience in carrying out preformulation studies with New Chemical Entities (NCEs). Capability to design and conduct preformulation studies for NCEs and to provide support to both API and formulation scientists is desired.

Patents – Job code PLSL-005

Ph.D/ M.S./ M.Pharm. in Organic Chemistry or Pharmaceutical Chemistry, with at least 1 - 2 years pharmaceutical industry experience in the following areas: chemical structure searches, particularly Markush structures, patent searches; drafting and filing patent applications, especially for new chemical entities (NCEs); patentability / infringement / freedom to operate analysis; and patent prosecution. Candidate should have good working knowledge of patent laws of different countries particularly India, USA and Europe, and excellent analytical and writing skills. A law degree or a postgraduate diploma in patent law will be an added advantage.

Discovery Informatics Scientists – Job code PLSL-006

The positions broadly involve theoretical and computational work at different stages of novel drug discovery research, including bioinformatics, cheminformatics, molecular modeling, biostatistics, computational statistics, mathematical modeling and/or computer programming. The job will involve one or more of the following: developing predictive ADMET models; implementing predictive ADMET software; QSAR modeling; Pharmacophore modeling; docking; virtual screening; structural bioinformatics; structure-based drug design; biostatistical analysis of pharmacological data; statistical/computational modeling of data; molecular dynamics simulations; creation and maintenance of scientific databases; data management; writing utility programs using shell, html, Perl, C++, Java, R, or vcl in MOE. Ideal candidates would be those with an M.S./Ph.D/ M.Tech / M.Pharm or exceptionally bright graduates in relevant areas, with a sound foundation in medicinal/organic chemistry, statistics, computer science or structural biology. Additional qualifications in bioinformatics or cheminformatics / molecular modeling would be preferred. The candidates must have a flair for using computers, including new software packages and be proficient/have hands-on experience in using Linux. Knowledge of modeling and assessing X-ray crystal structure models will be an asset. The minimum experience required for the positions available, is six-month training, in a relevant area.

Corporate Regulatory Affairs – Job code PLSL-007

The candidate should have an advanced degree in Sciences, with at least 1 year of experience in assembling data packages for submission for INDs, CTAs, etc, and be familiar with regulatory requirements for initiating first-in-man, as well as advanced clinical studies, in US and Europe.

Drug Development / Project Management Scientist – Job code PLSL-008

The candidate should have an M.S. degree but preferably a Ph.D in biological sciences or chemistry with experience in advancing a lead candidate from pre-clinical to clinical development. The ideal candidate should have successfully transitioned a new chemical entity from the discovery to the development phase. Specific responsibilities will include participation in multiple therapeutic area teams to provide assistance in drug development, design, execution and monitoring of non-GLP and GLP studies to support global development efforts. He or she should have excellent command of written and spoken English.

Business Development – Job code PLSL-009

The candidate should have a Ph.D. in biological sciences and/or a Masters degree in Business Administration. At least 6-10 years of relevant industry experience (business development role in a drug discovery and development organization) is a must. The job will involve initiating and formalizing strategic alliances with pharmaceutical and biotech companies, carrying out in licensing / out-licensing activities, technology evaluation and acquisition, agreements with industry and academic institutions. The candidate should have a strong understanding of drug discovery and development process, excellent communication and negotiation skills.

Evidence of scientific achievements and leadership through a strong publication and patent record, as well as excellent communication skills are required for most of the above positions. Candidates must possess the ability to work with cross-functional partners. If you wish to apply, kindly email us your resumé at research.recruitment@piramal.com indicating the specialization applied for along with the accompanying job code in the subject line.

Queens College

DEAN, MATHEMATICS & NATURAL SCIENCES DIVISION

The Division of Mathematics & Natural Sciences at Queens College of the City University of New York invites applications for the position of dean. The Division includes the departments of Biology, Chemistry & Biochemistry, Computer Science, Earth & Environmental Sciences, Family Nutrition & Exercise Sciences, Mathematics, Physics, and Psychology, as well as research centers such as the Center for the Biology of Natural Systems. The 2010 edition of the U.S. News and World Report *America's Best Colleges* guidebook ranks Queens among the top 10 public universities in its category. Our scenic 77-acre campus hosts over 16,000 undergraduates and over 4,500 graduate students. PhD candidates perform laboratory research on campus in a consortial arrangement with CUNY's Graduate Center and senior colleges, and many doctoral level courses are taught on campus. Under CUNY's Decade of Science initiative, Queens College is hiring aggressively in the sciences and expanding core facilities, and has just completed a new Chemistry addition. The dean works closely with department chairs and reports to the Provost.

The successful candidate will demonstrate a commitment to urban education and to a diverse faculty and student body, a collaborative and collegial management style, and experience with curriculum review and budget management. Please see complete details of position responsibilities and requirements at www.qc.cuny.edu/HR/Pages/JobListings.aspx.

Anticipated start date is August 25, 2010. Review of applications will begin on January 1, 2010 and continue until the position is filled. Applicants should submit a cover letter, CV, and contact information for three professional references, preferably via email to lisiansky@qc.cuny.edu, or to:

Dr. Alexander Lisiansky, Chair
Dean of MNS Division Search Committee
Queens College, CUNY
65-30 Kissena Blvd.
Flushing, NY 11367-1597
AA/EOE/IRCA/ADA



The Best Care -The Best Career

Veterans Affairs Medical Center San Francisco

Associate Chief of Staff for Research and Development, San Francisco VA Medical Center

The San Francisco VA Medical Center is seeking an M.D. or Ph.D. scientist to serve as the Associate Chief of Staff for Research and Development. Responsibilities include administration of the Medical Center's \$78 million research program, advocacy for the research enterprise and mentoring of junior research faculty. Applicants must have a well-established research program and must have demonstrated administrative skills. Academic appointment would be at the University of California San Francisco, School of Medicine. U.S. citizenship is required.

Interested applicants should submit their curriculum vitae and three professional references to Jia F. Li; Office of the Chief of Staff, (11D); 4150 Clement Street; San Francisco, CA 94121; 415-750-2185.

THE DEPARTMENT OF VETERANS AFFAIRS IS AN EQUAL OPPORTUNITY EMPLOYER



UNIVERSITY OF MINNESOTA, Twin Cities Department of Genetics, Cell Biology, and Development Tenure-Track Faculty Position in Cellular Dynamics and Imaging

The Department of Genetics, Cell Biology and Development is recruiting candidates for a tenure-track position at the Assistant Professor level. We seek investigators who are addressing fundamental questions concerning intracellular transport mechanisms using state of the art imaging and quantitative approaches, including computational methods. The department is recognized for its expertise in the structure/function of the cytoskeleton, the developmental genetics of model organisms (including worms, flies, zebra fish and mice), transposon biology and genome manipulation, human and cancer genetics, and chromosome instability. We have recently recruited faculty members with interests in cell cycle controls, chromosomal segregation, protein degradation, membrane trafficking, cell adhesion, and signaling. Competitive candidates will complement and extend existing departmental strengths, while building links to related translational research in neighboring clinical departments and institutes.

Candidates must have a Ph.D. and/or M.D. degree, at least three years of post-doctoral experience, a strong publication record, and they must also be U.S. citizens or be able to secure permanent resident status. Successful candidates will be expected to develop independent, externally funded research programs, interact collaboratively among a variety of disciplines, and participate in the undergraduate, graduate, and/or professional teaching programs of the Department. Starting salaries will be commensurate with education and experience, and competitive start-up packages are available. For additional information on the Department, see <http://www.gcd.umn.edu/>.

Individuals interested in this position should visit (<https://employment.umn.edu>) and search for requisition #164176. Follow instructions to Upload/Attach a CV and statement of research interests. In addition, three letters of recommendation that consider both research and teaching potential should be sent electronically as PDF files to: Mary Muwahid (email: muwahid001@umn.edu). Review of applications will begin December 15th, 2009 and will continue until the position is filled.

The University of Minnesota is an
Equal Opportunity Educator and Employer.



Faculty Positions University of Pittsburgh School of Medicine Department of Pharmacology and Chemical Biology

Applications are invited for tenure-stream Assistant Professor positions in the Department of Pharmacology and Chemical Biology. Candidates who have a Ph.D., M.D. or equivalent graduate degree are being recruited to expand a strong cadre of faculty.

The University of Pittsburgh School of Medicine currently ranks 6th among NIH-funded academic medical centers, and the department is consistently one of the top NIH-funded departments of pharmacology. The School of Medicine and the department are executing an exciting vision for both the expansion and integration of basic research, clinical investigation, and patient care missions. The department houses state-of-the-art facilities supporting microscopy, mass spectrometry, protein chemistry, gene expression profiling, genetic models of disease, receptor analysis, physiologic monitoring, physical chemistry, organic/combinatorial chemistry and high-throughput drug discovery.

Successful candidates must have excellent communications skills, develop an outstanding, extramurally supported research program, and be committed to the graduate and medical teaching missions of the department. Salary and benefits are highly competitive, with excellent start-up investments. Although all strong applicants will be considered, the department is especially interested in applicants with expertise in cell and organ systems pharmacology, cardiovascular pharmacology, neuropharmacology, endocrine pharmacology, cell signaling, or drug discovery.

Applicants should provide via e-mail a one-page statement of research objectives, curriculum vitae, and contact information for three references to: Bruce A. Freeman, Ph.D., Irwin Fridovich/UPMC Professor, Chair of Pharmacology and Chemical Biology, University of Pittsburgh, School of Medicine, W1340 Biomedical Science Tower, Pittsburgh, PA 15261; E-mail: pharmdev@pitt.edu.

The University of Pittsburgh is an
Affirmative Action, Equal Opportunity Employer.

DO YOU SEE YOURSELF AS A RESEARCHER?

More than 8 000 key people are committed daily to
developing and enhancing research into human health

124 tenure positions available for scientists m/f
dedicated to biomedical research

Inserm is the only French public research body entirely dedicated to human health. Its researchers are committed to studying all diseases, whether common or rare. Through its diversity of approaches, Inserm provides a unique environment for researchers. 13 000 researchers, engineers and technicians work in the 318 Inserm laboratories housed in hospitals, universities and research campuses, all over France. The positions of Research Associates and of Research Directors are open to PhD (or equivalent title) holders, without nationality restriction.

Application modalities:

visit our website: <http://www.eva2.inserm.fr>

Application deadline: January 7th, 2010 - 4.00 pm (GMT+1)

TENURE TRACK FACULTY POSITION IN STRUCTURAL BIOLOGY

The Program in Molecular and Translational Medicine (MTM) of the Fox Chase Cancer Center, Philadelphia, PA invites applications for a tenure-track (Assistant or Associate level) faculty position in structural biology. We are interested in outstanding individuals who have significant records of accomplishment. Areas of interest include X-ray crystallography, NMR spectroscopy, and other biophysical and computational approaches to problems in cancer biology. Candidates should have a focus on one particular area relevant to cancer research, including (but not limited to) cell signaling, DNA repair, cell cycle control, apoptosis, or translational medicine. The MTM program includes a spectrum of research addressing structural biology, cancer signaling networks, novel targeted therapeutics, and clinical trials (<http://www.fccc.edu/research/areas/mtm/index.html>). Individuals interested in opportunities for collaborative, multidisciplinary translational applications of their research are particularly encouraged to apply. Research at the Fox Chase Cancer Center is supported by state-of-the-art core facilities, extramural and intramural postdoctoral training grants, endowed fellowships, and marked by an outstanding funding record for investigator-initiated grant applications.

Candidates should send a curriculum vitae, statement of research interests, and names of three references to the head of the search committee, **Dr. Erica Golemis** at MTMsearch@fccc.edu. Fox Chase Cancer Center is an equal opportunity employer.

FOX CHASE
CANCER CENTER



Northeastern University

FOUNDING DEAN OF THE COLLEGE OF SCIENCE NORTHEASTERN UNIVERSITY BOSTON, MASSACHUSETTS

Northeastern University seeks an outstanding scholarly and entrepreneurial leader for the position of founding Dean for its newly established College of Science. Reporting to the Provost and Senior Vice President for Academic Affairs, the founding Dean will have an extraordinary opportunity to shape a new college that will enhance Northeastern University's distinctive educational mission and national and international reputation for joining translational with fundamental research in an interdisciplinary environment.

Located in the heart of Boston, in the arts district and the biomedical research corridor, Northeastern University is a private national research university offering a wide range of programs leading to degrees through the doctorate in eight schools and colleges. Over the past ten years, Northeastern has experienced extraordinary growth in admissions selectivity, research activity, campus life, donor support, and reputation. Today, Northeastern is the largest private research university in Boston proper, comprising more than 15,000 undergraduate and 5,000 graduate students and over 4,200 faculty and staff. The university operates with a \$798 million budget and expended \$55 million in research funding in FY 2009.

In 2006, under the leadership of newly appointed President Joseph Aoun, the University embarked on an institution-wide planning process that involved reflecting on the institution's purpose and envisioning its future. Today, with a new Mission Statement and Academic Plan in place, Northeastern is a vital, optimistic institution moving forward with confidence and energized by a sense of collective momentum and ambition. In line with its strategic goals, Northeastern has recently announced its decision to restructure the Colleges of Arts and Sciences and Criminal Justice and create a new College of Science, as well as Colleges of Arts, Media and Design, and Social Science and Humanities. This reorganization will better align academic departments that share educational and research goals and approaches, enabling them to advocate more effectively for resources and to project a clearer, unified identity to the outside world. In addition, the University is implementing a new hybrid budgetary model based on responsibility based management that will provide its deans with heightened autonomy, financial control, and decision-making authority.

This is an unusual and exciting opportunity to join a university that is on a dramatic upward trajectory, led by a strong executive leadership team, and moving to a management structure and style that empowers deans. Northeastern University seeks a Dean of Science with exemplary academic achievements, together with strategic leadership ability; outstanding interpersonal and communication skills; entrepreneurial spirit and high energy; and a passion for the unique mission of the College and University. In collaboration with the faculty of the College, s/he will articulate a vision and strategic plan that will strengthen interdisciplinary connections across the department units, the other colleges of the university, and Northeastern's science disciplines and the scientific, technological, and medical institutions of the Greater Boston region.

Northeastern University has retained Isaacson, Miller, a national executive search firm, to assist in this recruitment. Review of candidates will begin immediately and continue until an appointment is made. Applications, including cover letter, vita, and three references, should be submitted in electronic form, to: **Vivian Brocard, Vice President, Nureen Das, Associate, Isaacson, Miller, E-mail: 3938@imsearch.com. Electronic applications are strongly encouraged.**

*Northeastern University is an Equal Opportunity Employer.
Persons of color and women are encouraged to apply.*

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UNIVERSITY OF MICHIGAN MEDICAL SCHOOL



Fully eight percent of the human genome is made up of retroviruses that have inserted themselves into our genetic material, but very little is known concerning the role of these endogenous retroviruses in modern biology and disease.

Our laboratory has recently received substantial grant support to study how one of these endogenous retroviruses, HERV-K, might replicate and participate in the pathogenesis of cancers, such as lymphoma, and in HIV pathogenesis (Contreras-Galindo, R., Kaplan, M. H., Leissner, P., Verjat, T., Ferlenghi, I., Bagnoli, F., Giusti, F., Dosik, M. H., Hayes, D. F., Gitlin, S. D., and Markovitz, D. M., Human endogenous retrovirus K (HML-2) elements in the plasma of people with lymphoma and breast cancer. *J Virol* 82:9329-9336, 2008).

We are looking for ambitious postdoctoral fellows to join in this project. A background in molecular biology is an important qualification, but scientists from any discipline are invited to apply.

To submit an application, please send a copy of your curriculum vitae and a list of three references with their contact information to **Professor David Markovitz, Department of Internal Medicine, University of Michigan (dmarkov@umich.edu)**.



Assistant/Associate Professor Neuroscience

The Institute of Neurobiology of the University of Puerto Rico Medical Sciences Campus seeks applicants for two tenure-track positions, one at the Assistant, and the other at the Assistant or Associate Professor level. Candidates whose research uses simple model systems to understand fundamental problems in neuroscience, or those who focus on the relationship between neural plasticity and the environment will receive highest consideration, although all fields of quality neuroscience research are encouraged to apply. The candidate should have a doctoral degree, a minimum of two years postdoctoral training, research productivity commensurate with experience and demonstrate commitment to developing an independent research program. Both positions will be housed at the Institute of Neurobiology; one is a full-time research position that carries no teaching obligations, and the other will be affiliated with the Dept. of Anatomy and Neurobiology at the University of Puerto Rico's Medical Sciences Campus. Neuroscience has been targeted by UPR as a special area of growth, and this is reflected in both infrastructure investment and faculty hiring. The successful candidates will receive competitive start-up packages and laboratory space in the heart of historic Old San Juan. In a gesture that is both symbolic and substantive, the newly enacted Law 101 of the Commonwealth of Puerto Rico gives investigators with competitive U.S. funding substantial tax breaks on their personal income.

Send curriculum vitae, a description of research interests, the names and contact information of three referees, and reprints of two significant recent publications to: **Dr. Joshua Rosenthal, Search Committee, Institute of Neurobiology, 201 Boulevard del Valle, San Juan, PR 00901**. In order to receive full consideration, applications should be received by **January 15, 2010**.

For more information please contact **Dr. Joshua Rosenthal** at joshua.rosenthal@upr.edu.

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TEXAS A&M
UNIVERSITY

Department of Biochemistry and Biophysics

The Department of Biochemistry and Biophysics at Texas A&M University (<http://biochemistry.tamu.edu>) invites applications for tenure-track faculty positions in an open search with an emphasis on areas related to metabolomics, chemical genetics and drug discovery. Faculty rank is open and applications from candidates at all levels are encouraged. In addition to establishing vigorous independent research programs, the successful candidates will fully participate in our undergraduate and graduate educational programs.

Candidates should submit a curriculum vitae, up to three reprints, a description of research plans of up to three pages, and arrange for three professional reference letters to be submitted. All documents can be submitted electronically to tamu@bichsearch.tamu.edu or in paper form to:

**Biochemistry Search Committee
Texas A&M University
Department of
Biochemistry and Biophysics
2128 TAMU
College Station, TX 77843-2128**

Review of applicants will begin **January 15, 2010** and will continue until the positions are filled.

Texas A&M University is an Equal Opportunity/Affirmative Action Employer that is committed to improving diversity.

UNIVERSITY OF WYOMING

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Obtaining a Masters or Doctoral degree in Neuro-science from the University of Wyoming offers a uniquely personalized approach to mentoring students, breadth of research activities from molecular mechanisms to pre-clinical studies, and a means of assuring student professional success after matriculation.

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- Neuroendocrinology
- Neuropharmacogenomics
- Sensory neurophysiology
- Behavioral neuroscience
- Biomimetic machine vision
- Neural development and neurogenesis

Students have access to an outstanding Microscopy Facility and a Macromolecular Core Facility. The program is an integral partner in the Front Range Neuroscience Group, a thriving chapter of the Society of Neuroscience.

To apply online or learn more, visit neuroscience.uwyo.edu or email neuroscience@uwyo.edu

The University is a Carnegie Foundation Research/Doctoral Extensive Institution, and is an AA/EEO employer.

ECOLOGIST - Vassar College

The Department of Biology at Vassar College invites applications for a tenure-track faculty position at the level of assistant professor beginning Fall 2010. We seek an ecologist whose research and teaching interests may include but need not be limited to ecosystems and computational or quantitative ecology. Candidates should demonstrate the ability to integrate across several levels of biological organization in their teaching and research. The successful candidate is expected to develop an upper-level course in his/her specialty as well as teach at the intermediate and introductory levels. The College has a rich tradition of multidisciplinary and interdepartmental teaching, and we encourage the successful candidate to participate in programs such as Environmental Studies. Development of a productive research program with student participation is expected, and start-up funding and personal laboratory space are provided. A Ph.D. is required and postdoctoral experience is preferred. Applicants should submit a curriculum vitae, representative reprints, a statement of research interests and goals, a statement of teaching interests and philosophy, and three letters of reference. To apply visit <http://deanofthefaculty.vassar.edu/positions/>. For information on Vassar's Biology Department visit <http://biology.vassar.edu/>. Consideration of applications will begin on **January 18, 2010**.

Vassar College is an Equal Opportunity/Affirmative Action Employer and is strongly and actively committed to diversity within its community. Applications from members of historically underrepresented groups are especially encouraged.



COLUMBIA UNIVERSITY

IN THE CITY OF NEW YORK

THE 2010 LOUISA GROSS HORWITZ PRIZE FOR BIOLOGY OR BIOCHEMISTRY

The Louisa Gross Horwitz Prize was established under the will of the late S. Gross Horwitz through a bequest to Columbia University and is named to honor the donor's mother, Louisa Gross Horwitz was the daughter of Dr. Samuel David Gross (1805-1889), a prominent surgeon of Philadelphia and author of the outstanding *Systems of Surgery* who served as President of the American Medical Association.

Each year since its inception in 1967, the Louisa Gross Horwitz Prize has been awarded by Columbia University for outstanding basic research in the fields of biology or biochemistry. The purpose of this award is to honor a scientific investigator or group of investigators whose contributions to knowledge in either of these fields are deemed worthy of special recognition.

The Prize consists of an honorarium and a citation which are awarded at a special presentation event. Unless otherwise recommended by the Prize Committee, the Prize is awarded annually. Dr. Victor Ambros, University of Massachusetts Medical School, Worcester, MA, and Dr. Gary Ruvkun, Harvard Medical School, Boston, MA, were the 2009 awardees.

QUALIFICATIONS FOR THE AWARD

The Prize Committee recognizes no geographical limitations. The Prize may be awarded to an individual or a group. When the Prize is awarded to a group, the honorarium will be divided among the recipients, but each member will receive a citation. Preference will be given to work done in the recent past.

Nominations must be submitted electronically at: <http://www.cumc.columbia.edu/horwitz/>

Nominations should include:

- 1) A summary, preferably less than 500 words, of the research on which this nomination is based.
- 2) A summary, preferably less than 500 words, of the significance of this research in the fields of biology or biochemistry
- 3) A brief biographical sketch of the nominee, including positions held and awards received by the nominee
- 4) A listing of up to ten of the nominee's most significant publications relating to the research noted under item 1.
- 5) A copy of the nominee's curriculum vitae.

Deadline date: January 31, 2010

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DREW UNIVERSITY

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The Residential School on Medicinal Chemistry is a weeklong graduate level course organized to provide an accelerated program for medicinal chemists, biologists and other industrial and academic scientists who wish to broaden their knowledge of small molecule drug discovery and development. The School's aim is to concentrate on the fundamentals that are useful in drug discovery spanning initial target validation through clinical development. Several case histories of recent successful drug development programs will also be presented.

The five-day program consists of lectures, seminars, case histories and discussions covering the following topics:

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• Receptor Binding Assays	• Drug-like Properties
• Enzyme Inhibition	• Plasma Protein Binding
• Ion Channel Drugs	• Pharmacokinetics
• G-protein Coupled Receptors	• Drug Metabolism
• Kinase Inhibitors	• Preclinical Toxicology
• Lead Discovery & Modification	• Clinical Development
• Structure-based Drug Design	• Drug Discovery Strategies

William Greenlee, Vincent Gullo and Kenneth Thomas, *Co-organizers*

More information and application forms can be obtained at
www.drew.edu/depts/resmed.aspx or by contacting the
School's office at Drew University, Phone: 973/408-3787;
Fax: 973/408-3504 or email: resmed@drew.edu

POSITIONS OPEN

VANDERBILT UNIVERSITY

FACULTY POSITION

The Division of Cardiovascular Medicine invites applications for a tenure-track faculty position. We seek top candidates using cutting-edge approaches in the broad area of basic cell and developmental biology of the cardiovascular system to fill a position at the rank of **ASSISTANT, ASSOCIATE or FULL PROFESSOR**. The successful candidate will receive a substantial startup package commensurate with the career stage of the appointee and will be housed in newly constructed/renovated facilities. The appointee will also benefit from a broad array of state-of-the-art core facilities, active graduate and postdoctoral programs, as well as the interdisciplinary and interactive environment of Vanderbilt University. Complete applications should include curriculum vitae, a brief statement of future research plans, and three letters of recommendation, solicited by the applicant. Deadline for receipt of full application materials is February 1, 2010.

Address all inquiries and materials to:

Dr. David Bader
Chair Faculty Search Committee
Division of Cardiovascular Medicine
346 Preston Research Building
2220 Pierce Avenue
Nashville, TN 37232-6300 U.S.A.
E-mail: david.bader@vanderbilt.edu

Vanderbilt University is an Equal Opportunity/Affirmative Action Employer. Applications from women and people from diverse racial, ethnic, and cultural backgrounds are encouraged.

STANFORD UNIVERSITY SCHOOL OF MEDICINE. Department of Microbiology and Immunology invites applications for a tenure-track faculty position at the **ASSISTANT PROFESSOR** level. We are looking for highly interactive candidates with an outstanding record of research achievement in the area of innate or acquired immunity, virology, or parasitology with an emphasis on the molecular mechanisms of pathogenesis. The predominant criteria for appointment in the University tenure track are a major commitment to research and teaching.

The Department of Microbiology and Immunology (website: <http://microimmuno.stanford.edu>) is a collegial, interactive, and interdisciplinary environment that spans research areas of bacteriology, virology, parasitology, and immunology. Current faculty use a variety of classical and modern approaches to study aspects of host-microbe interactions. Applicants are expected to establish a vigorous and innovative research program that complements and extends the current research of the Department. Responsibilities will also include teaching graduate, undergraduate, and medical students.

Interested applicants should submit curriculum vitae, a statement of research objectives, and a statement of teaching preferences. Please arrange for three letters of reference to be sent directly to:

Peter Sarnow, Ph.D.
c/o Mayumi Beppu
Department of Microbiology and Immunology
Stanford University School of Medicine
Fairchild Science Building
299 Campus Drive, D338c
Stanford, CA 94305-5124

Applications will be accepted until January 15, 2010. *Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women and members of minority groups, as well as others who would bring additional dimensions to the University's research, teaching, and clinical missions.*

POSITIONS OPEN

OAKLAND UNIVERSITY DEPARTMENT OF BIOLOGICAL SCIENCES TENURE-TRACK FACULTY POSITION (ASSISTANT PROFESSOR) IN ENGINEERING BIOLOGY/ BIOENGINEERING

The Department of Biological Sciences at Oakland University invites applications for a tenure-track **ASSISTANT PROFESSOR** position to be filled by August 2010. Research areas of particular interest include but are not limited to: biotechnology, molecular genetic engineering, or tissue engineering. A Ph.D. and postdoctoral experience are required as well as a strong research track record evidenced by publications. Lab space and competitive start up package will be provided to help establish a successful research program. The person hired is expected to develop a vigorous, extramurally funded research program, establish ties with local and national industry leaders, and mentor graduate students in doctoral programs. Together with our School of Engineering and Computer Sciences, the Department offers a new B.S. Engineering Biology program. The successful candidate is expected to have a role in the program's continued development and to engage with the majors via classroom instruction and project mentorship.

The Department of Biological Sciences at website: <http://www2.oakland.edu/biology/> is a modern, well-equipped, research oriented department with active graduate programs at the M.S. and Ph.D. level. Oakland University is a state-supported institution of 19,000 students situated on a beautiful 1,500-acre campus 25 miles north of Detroit. Opportunities exist for clinical collaborations through the recently established Oakland University William Beaumont School of Medicine.

Applicants should mail their curriculum vitae, statement of research plans and teaching philosophy, copies of key reprints, and have three letters of recommendation forwarded by February 10, 2010 to: **Arik Dvir, Engineering Biology Search Chair, Department of Biological Sciences, Oakland University, Rochester, MI 48309-4401**. Questions should be addressed to e-mail: dvir@oakland.edu. *Oakland University is an Equal Opportunity Employer.*

SENIOR STAFF SCIENTISTS

The Henry M. Jackson Foundation (HJF) is seeking two senior staff **IMMUNOLOGISTS** to support the Enteric Diseases Department, Naval Medical Research Center, Silver Spring, Maryland. Must have expertise in bacterial vaccinology and mucosal immunology. The Department serves as a central research hub for the development of bacterial enteric vaccines within the U.S. Military Infectious Diseases Research Program. Will serve as principal investigators in the discovery and development of innovative vaccines against diarrheagenic *E. coli* (ETEC) or *Campylobacter jejuni* in intramural programs led by **Drs. Stephen Savarino and Patricia Guerry**. Will directly supervise research groups to evaluate novel vaccine candidates in small animals, develop assays to assess immune responses and explore protective immunity, and provide immunology support in early clinical phase vaccine trials. Will pursue independent investigations, collaborations, and outside funding opportunities to study basic aspects of immunology of ETEC or *C. jejuni*. Must have a Ph.D. in immunology, microbiology, or related field with three to five years of relevant postdoctoral research experience including assessment of immune responses to mucosal pathogens, a strong animal research background, and successful assay development. Please apply online at website: <http://www.hjf.org/careers>. Click Advanced Search and enter job number 204937 in the Job Opening ID box. Or transmit your resume to fax: 240-314-7334. Please specify title and job number on fax.

POSITIONS OPEN

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Postdoctoral positions funded by an NCI training grant are available for *U.S. citizens or permanent residents*. Applicants must directly contact a member of the training faculty prior to submitting an application, which will include a letter of interest, curriculum vitae, and long-term research goals. For information on trainers see website: <http://www.unmc.edu/Eppley/crgpfaculty.htm> or contact: **Diane Petersen, Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE 68198-6805**. After contacting a member of the training faculty, you may apply to position #3905 at website: <https://jobs.unmc.edu/applicants>.



POSTDOCTORAL POSITION (100 percent, multiyear) in mouse genetics and neurodegenerative disease available immediately at University of California, Davis to use and generate mouse models to study the genetic, cellular, molecular, and mitochondrial mechanisms of neurodegenerative disease. Expertise in cloning, knockouts, and conditional gene expression as well as molecular and biochemical experience are required. Salary depends upon experience (NIH scale). Send curriculum vitae to e-mail: gcortopassi@ucdavis.edu. Website: <http://cortopassilab.ucdavis.edu/>. *UCD is an Affirmative Action/Equal Opportunity Employer.*

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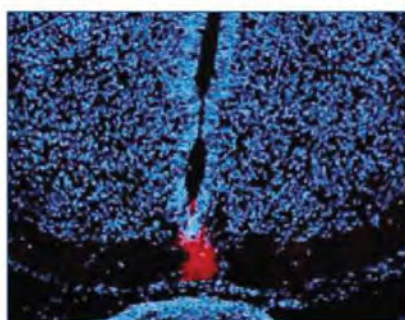
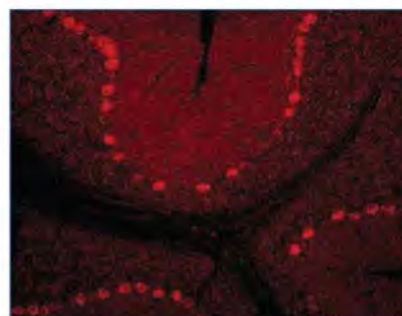
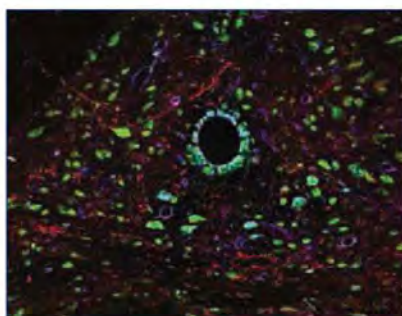
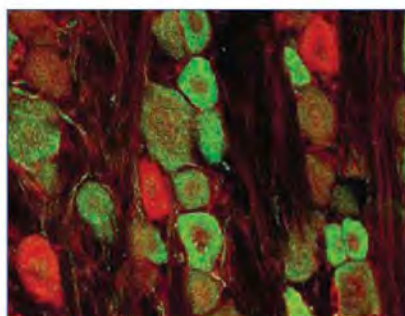
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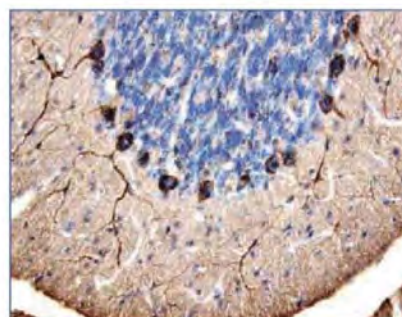
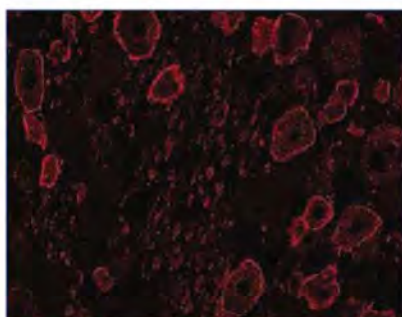
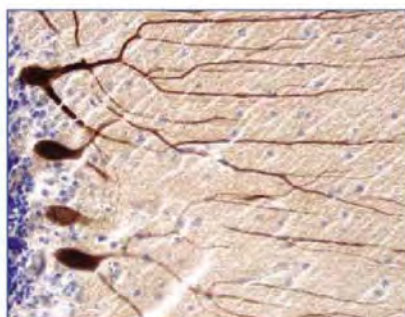
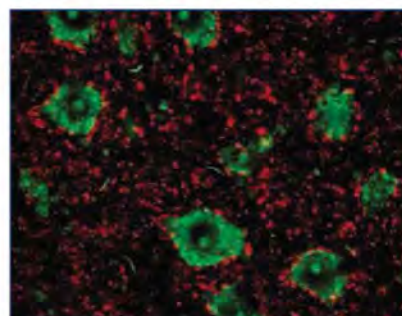
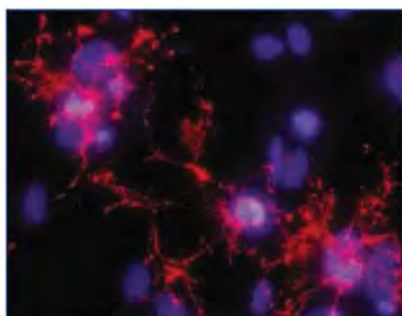
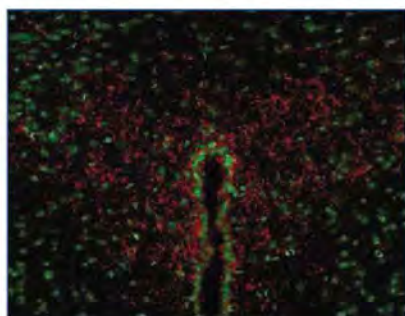
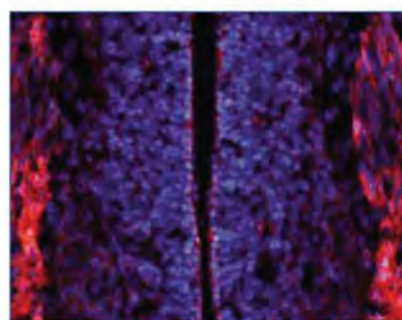


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